Drug interactions and the statins

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Abstract

DRUG INTERACTIONS COMMONLY OCCUR in patients receiving treatment with multiple medications. Most interactions remain unrecognized because drugs, in general, have a wide margin of safety or because the extent of change in drug levels is small when compared with the variation normally seen in clinical therapy. All drug interactions have a pharmacokinetic or pharmacodynamic basis and are predictable given an understanding of the pharmacology of the drugs involved. Drugs most liable to pose problems are those having concentration-dependent toxicity within, or close to, the therapeutic range; those with steep dose–response curves; those having high first-pass metabolism or those with a single, inhibitable route of elimination. Knowing which drugs possess these intrinsic characteristics, together with a knowledge of hepatic P-450 metabolism and common enzyme-inducing and enzyme-inhibiting drugs, can greatly assist physicians in predicting interactions that may be clinically relevant. This article reviews the pharmacology of drug interactions that can occur with hydroxymethylglutaryl – coenzyme A (HMG–CoA) reductase inhibitors (statins) to illustrate the scope of the problem and the ways in which physicians may manage this important therapeutic class of drugs.

Background

All important drug interactions, with the possible exception of idiosyncratic or allergic reactions, have a pharmacokinetic or pharmacodynamic basis, or both.[1,2] Pharmacokinetic interactions refer to those where drugs or other factors cause an alteration in the concentration of unbound drug acting on the tissues. They include interactions that may lead to changes in drug absorption, drug distribution (either through binding to plasma proteins or, more importantly, binding and uptake into tissues) and drug elimination. Pharmacodynamic interactions refer to those where changes occur in tissue sensitivity or response to the same unbound drug concentrations.

The consequences of a drug interaction depend upon patient-related as well as drug-related factors (Fig. 1).[3] These include the magnitude and direction of the concentration or effect changes, as well as the steepness and separation of the dose–response of the drug's intended (therapeutic) and unintended (adverse) pharmacologic actions.[1] Large changes in the concentration or tissue response to a drug possessing a flat dose–response relation or low intrinsic toxicity may be of little clinical importance. Alternatively, small changes in the concentration of potent or highly toxic drugs can be disastrous. Individual susceptibility to adverse drug effects because of health- (e.g., age, pregnancy) and disease-related factors (e.g., renal, hepatic, CNS) should also be considered. As well, the body may minimize a drug's effect through offsetting changes in tissue sensitivity, by up-regulation or down-regulation of receptor numbers or by changes in receptor–effector coupling, or both.[4] What might produce minimal impairment on one occasion could be incapacitating on another occasion or in a less tolerant individual.

Interactions between drugs binding to the same sites on plasma proteins are rarely associated with changes in drug response.[1,2] The reason for this is that most of the drug exists in the body in tissue stores, mainly in muscle and fat, not in the circulation. Thus, even large decreases in the amount of drug bound to plasma proteins is effectively buffered by a greater distribution in peripheral tissues with little or no change in unbound concentrations. The one exception occurs with drugs possessing

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small distribution volumes, like warfarin, where binding interactions confined largely to the circulation produce large changes in unbound concentrations and drug effects. What is important to remember is that laboratories usually report total drug concentrations and not unbound drug concentrations. Therefore, target ranges of clinically monitored drugs should be adjusted downwards in the presence of a binding interaction, normally with no change in dosage. Similar considerations apply if the levels of albumin or other binding proteins are not within the expected range.

In contrast, displacement from tissue-based binding sites or the inhibition of carrier-mediated uptake into tissues can produce large changes in unbound drug concentrations.[2] Drugs and their metabolites move out of tissues as readily as they move in, and muscle and fat often contain large body stores, particularly following multiple dosing. The factors causing redistribution from tissues into the circulation are not well understood, although evidence suggests that this occurs commonly with lipophilic drugs that have large distribution volumes.[5] Examples of clinically relevant interactions involving the inhibition of drug distribution and transport include the 2- to 3-fold elevations in digoxin serum concentrations following the concomitant administration of quinidine or verapamil.[6]

The inhibition or induction of hepatic drug metabolism is a major source of variability in drug response and is the basis of many adverse drug interactions.[7] Paramount to an understanding of this is a consideration of the role of the liver in the overall elimination of the drug. Most drugs are removed from the body through multiple competing pathways of renal and hepatic excretion. If one or several of these become blocked because of disease or the action of another drug, clearance will diminish, dependent upon the relative contribution of the affected pathway(s) to the total elimination of the drug.[8] If this occurs, steady-state concentrations and, correspondingly, drug or adverse effects rise. However, these in turn drive elimination through other pathways as long as they are unencumbered. Therefore, drugs that have few or minor alternative pathways are particularly prone to large concentration increases when elimination is impaired.

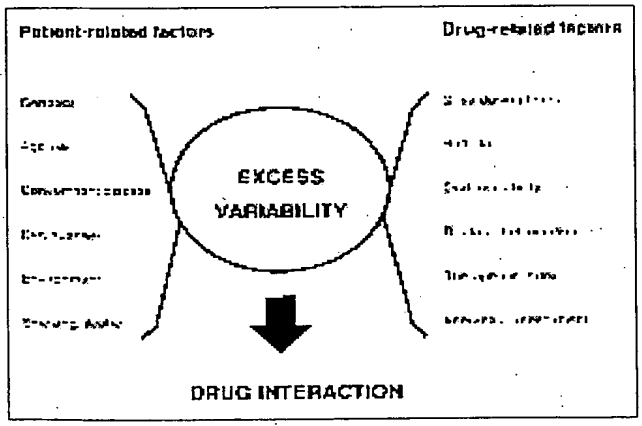

Fig. 1: Factors influencing drug interactions. (Adapted from Hansten).[3]

First-pass metabolism by the gut and liver is another important consideration. If a drug has low oral availability due to high presystemic elimination, there may be large increases in the amount of drug getting into the body if metabolism is inhibited. Where the parent drug is inactive and the pathway normally results in the formation of an active metabolite, drug response may diminish rather than increase when metabolism is inhibited.[9] Conversely, response may be unchanged if both parent and metabolites are active — increases in the concentration of the parent offset by decreases in the metabolites.[9]

The mechanism of interaction is also an important factor; an interacting drug may not be a known inhibitor but merely a substrate for the same metabolic pathway and thereby produce only minor dose-dependent competition at the active enzyme site.[10] In this case, the affinity of the substrate for the enzyme and the unbound concentration and half-life of the inhibiting drug are important determinants of the extent and time course of the interaction. Alternatively, inhibition may be noncompetitive or uncompetitive, wherein the effect is likely to be more complete and long lasting, requiring resynthesis of new enzyme before it can be overcome.[10]

The cytochrome P450 superfamily

Hepatic metabolism is served by a superfamily of oxygenases known as the cytochrome P450s. The purpose of these enzymes is to add a functional group to a drug, an environmental chemical or an endogenous molecule and, in so doing, increase either its polarity and excretion from the body or its interaction with similar enzymes. The most distinguishing characteristic of the cytochrome P450 family is its great diversity; members have a broad and overlapping substrate specificity and an ability to interact with almost any chemical species. The superfamily, referred to as the CYP enzymes, is subdivided according to the degree of homology in amino acid sequences. CYP enzymes possessing more than 40% homology are grouped together into families, which are designated by an Arabic numeral (e.g., the CYP1 family). Families are further divided into subfamilies, which are designated by a letter after the number (e.g., CYP2C and CYP2D subfamilies); members of each subfamily have more than 55% homology with one another. Finally, individual members are given an additional number (e.g., CYP3A4) to identify a specific enzyme pathway. Over 70 CYP families have been identified to date, of which 14 are known to occur in all mammals.[11] Of the 26 mammalian subfamilies, the CYP2C, CYP2D and CYP3A subfamilies are involved in the metabolism of most clinically relevant drugs. Important substrates, inducers and inhibitors of the major CYP enzymes are listed in Table 1.

The CYP2C subfamily comprises about 20% of all of the cytochrome P450s in the liver.[12] At least 6 different CYP2C isozymes have been characterized, each having greater than 80% homology with distinct but overlapping

Table 1: Inducers and inhibitors of major CYP enzymes

Enzyme; substrate	Enzyme inducers	Enzyme inhibitors
CYP1A2		
TCAs	Omeprazole, lansoprazole	Fluvoxamine (other SSRIs weak)
Haloperidol, olanzapine	Phenobarbital, phenytoin, carbamazepine	Ciprofloxacin (other quinolones weak)
Propranolol, local anesthetics	Erythromycin, clarithromycin, rifampin	Cimetidine
Theophylline, caffeine	Cigarette smoke	Isoniazid
Diazepam, chlordiazepoxide	Ritonavir	Oral contraceptives
Estrogens, tamoxifen	Insulin	Ticlopidine
CYP2C9		
ASA and most NSAIDs	Rifampin	Fluvoxamine (other SSRIs weak)
Phenobarbital, phenytoin	Phenobarbital, phenytoin, carbamazepine	Amiodarone
S-Warfarin, dicumarol		Omeprazole
Losartan (activation)		Ritonavir
Tolbutamide, sulfonamides, dapsone		HMG-CoA reductase inhibitors
Zidovudine		Tolbutamide
Diazepam, temazepam		Cimetidine (weak)
Fluoxetine, meclobemide		Azole antifungals (weak)
CYP2C19		
TCAs	Rifampin	Fluoxetine, fluvoxamine, paroxetine
Diazepam, temazepam	Phenobarbital, phenytoin, carbamazepine	Omeprazole, lansoprazole
Omeprazole, lansoprazole	Prednisone	Ritonavir
Propranolol	Norethindrone	Azole antifungals (weak)
Phenytoin, barbiturates, valproic acid		Cimetidine (weak)
Zidovudine		Ticlopidine
CYP2D6		
TCAs, SSRIs, venlafaxine		Quinidine
Phenothiazines, haloperidol		Fluoxetine, paroxetine, sertraline
Several β-blockers		TCAs, venlafaxine
Codeine, oxycodone, hydrocodone		Phenothiazines, haloperidol, nefazodone
Dextromethorphan		Ketoconazole
Omeprazole		Cimetidine
Halothane		Ritonavir
MDMA (ecstasy)		HMG-CoA reductase inhibitors
Encainide, flecainide, propafenone		Amiodarone, encainide
Selegiline		Chlorpheniramine
CYP2E1		
Acetaminophen	Ethanol	Disulfiram
Ethanol and other alcohols	Isoniazid	Ethanol
Inhalational anesthetics	Clofibrate	Cimetidine
Sulfonamides, dapsone		Isoniazid
CYP3A4		
Halothane	Phenytoin, barbiturates	Ketoconazole, itraconazole, fluconazole
Fentanyl, alfentanil, sufentanil	Rifampin	Erythromycin, clarithromycin
TCAs, SSRIs	Erythromycin	TCAs, nefazodone, venlafaxine
Erythromycin, clarithromycin	Omeprazole, lansoprazole	Fluvoxamine, fluoxetine, sertraline
HIV protease inhibitors	Dexamethasone, sex steroids	Cyclosporine, tacrolimus
Calcium-channel blockers (not diltiazem)	Cyclophosphamide	Omeprazole, lansoprazole
Lovastatin, simvastatin, atorvastatin		Calcium-channel blockers (esp. diltiazem)
Cyclosporine		Midazolam
Terfenadine, astemizole, loratadine		Corticosteroids
Midazolam, alprazolam, triazolam		Grapefruit juice
Cisapride		Tamoxifen

Note: TCA = tricyclic antidepressant, SSRI = selective serotonin reuptake inhibitor, HMG–CoA = hydroxymethylglutaryl – coenzyme A.

substrate specificity. Prostaglandins and sex steroids are important endogenous substrates of the CYP2C subfamily. The most abundant enzyme in this subfamily, CYP2C9, is responsible for the breakdown of a number of drugs including ASA and many of the nonsteroidal anti-inflammatory drugs, sulfonamides, phenytoin and S-warfarin (the more active enantiomer of warfarin). CYP2C19 is involved in the metabolism of diazepam, omeprazole and the tricyclic antidepressants. Both CYP2C9 and CYP2C19 are polymorphic, meaning the expression of these enzymes is under strong genetic influence and some individuals have markedly deficient activities. Indeed, 3% of white people and 20% of all those of Japanese descent lack CYP2C19 and are unable to metabolize diazepam and omeprazole by the usual pathways.[13,14] However, since many of the enzymes in this family have overlapping substrate specificities, it is unusual to see excessive or adverse drug effects even in people completely deficient in CYP2C19.[15] Serious interactions occur predominantly with drugs that have a low therapeutic index such as warfarin or phenytoin.[10]

CYP2D6 accounts for only 4% of hepatic CYP enzymes,[12] but is more unique in its metabolic profile. Important substrates for this enzyme include tricyclic antidepressants, selective serotonin reuptake inhibitors, neuroleptics, opioid analgesics and several of the β-adrenergic blockers. Seven to 10% of white people and 3% of black and oriental people are known to be deficient in the CYP2D6 enzyme, the so-called sparteine–debrisequine, poor metabolizer polymorph.[13,14] These individuals show great variability in clinical response (up to 1000-fold) and commonly have adverse effects to standard doses of drugs metabolized by this enzyme. Also, they are unable to convert codeine, oxycodone and hydrocodone to their active metabolites[16] and thereby derive little or no analgesic benefit from oral morphine analogues. Levels of CYP2D6 are not affected by age, sex or smoking status.[17] Inhibitors are quinidine, ketoconazole and most antidepressants and neuroleptics, and there are no known inducers of this enzyme.

The CYP3A subfamily, like CYP2D6, is involved in the metabolism of a large number drugs and other chemicals and is involved in many drug–drug and drug–food interactions. It is the most abundant of all of the P450s in the human liver (25%–28%, but sometimes as high as 70%) and is widely expressed throughout the gastrointestinal tract, kidneys and lungs.[12] More than 150 drugs are known substrates of CYP3A4, the major CYP3A isozyme, including many of the opiate analgesics, steroids, antiarrhythmic agents, tricyclic antidepressants, calcium-channel blockers and macrolide antibiotics. Although several substrates show age-dependent reductions in elimination, the enzyme itself does not appear to be altered.[18] Also, sex-related effects are small and probably not important. Ketoconazole, itraconazole, erythromycin, clarithromycin, diltiazem, fluvoxamine, fluoxetine, nefazodone, cyclosporine and dihydroxybergamottin and various substances found in grapefruit juice, green tea and other foods are potent inhibitors of CYP3A4 and are known to be responsible for many drug interactions.[10,15] Terfenadine, astemizole, cisapride, cyclosporine and many of the hydroxymethylglutaryl – coenzyme A (HMG–CoA) reductase inhibitors are potentially toxic drugs or drugs susceptible to large changes in concentration following enzyme inhibition and, therefore, are candidates for serious interactions with other substrates of CYP3A4.[10] These interactions can have serious clinical consequences.

Interactions with HMG–CoA reductase inhibitors

The HMG–CoA reductase inhibitors (statins) are associated with 2 uncommon but important side effects, namely asymptomatic elevation in liver enzymes and skeletal muscle abnormalities, which can range from benign myalgias to myopathy (10-fold elevation in creatine kinase with muscle pain or weakness) and life-threatening rhabdomyolysis.[19,20] The incidence of myopathy in patients taking statins alone is estimated to be 0.1%–0.2%,[20,21] and rhabdomyolysis is exceedingly rare. Evidence suggests that myopathy is a direct consequence of HMG–CoA reductase inhibition[22,23] and is dose-dependent.[24–27] Myopathy is most likely to occur when statins are administered with other drugs or chemicals that are themselves myotoxic or that elevate the concentrations of the statin to the toxic range. Indeed, the incidence of muscle disorders increases over 10-fold when statins are given with gemfibrozil,[20,28–31] niacin,[20,32] erythromycin,[33] itraconazole,[34,35] cyclosporine,[20,36,37] and diltiazem[38] among others.

Six statins are currently marketed for the treatment of dyslipidemia in North America. Lovastatin, simvastatin, atorvastatin and cerivastatin are all substrates of CYP3A4[39–41] and would be subject to marked inhibition of metabolism by azole antifungal agents, macrolide antibiotics, selective serotonin reuptake inhibitors, cyclosporine, diltiazem and grapefruit juice. Fluvastatin is metabolized by CYP2C9; it would not be affected by these substrates, but rather would have a different spectrum of interactions,[32,42] perhaps less clinically relevant because of the overlap between CYP2C isozymes. Pravastatin is not significantly metabolized by CYP and would be comparatively devoid of these effects.[43,44] Lovastatin, simvastatin and atorvastatin are all extensively metabolized on first-pass through the liver[39,40,45] with resultant low oral availability (5%–10%), whereas cerivastatin has an intermediate availability of around 60%.[46] Moreover, the active CYP3A metabolites of atorvastatin and cerivastatin contribute in large measure to their overall clinical activity.[40,46] Thus, inhibition of first-pass metabolism of lovastatin or simvastatin could result in 10–20 fold elevations (oral availability increasing from 5% to 100%) in steady-state concentrations with a marked liability to drug toxicity. Inhibition of metabolism of atorvastatin and cerivastatin, on the other hand, is likely to produce a balanced inhibition with small changes in the total

active drug concentration within the normal dosing range. Indeed, pharmacokinetic interactions of these types have been confirmed recently for each of the marketed statins.[47–53]

A MEDLINE review of all interactions involving a statin and any other drug between 1984 and 1999 revealed 1 case report of rhabdomyolysis in a patient receiving pravastatin and fenofibrate, but 27 cases of rhabdomyolysis in patients on simvastatin combined with either gemfibrozil, nefazodone, cyclosporine, itraconazole or mibefradil and 37 cases in those on lovastatin plus gemfibrozil, niacin, cyclosporine, itraconazole or erythromycin (references available on request). There are numerous other reports documenting lesser degrees of myopathy, myalgia and asymptomatic elevations in creatine kinase showing the same pattern of predilection for lovastatin and simvastatin. However, the mere potential for a drug interaction to occur, even its citation in the literature, provides little indication of the true incidence of adverse outcomes in routine clinical use. Monotherapy with lovastatin, pravastatin and simvastatin has a proven record of safety and efficacy in large clinical trials.[21,54,55] Moreover, there are numerous reports in the recent literature documenting the safe use of low dose statin–cyclosporine and statin–fibrate combinations in high-risk patients or patients with complex dyslipidemias[56,57] (other references available on request). Indeed, patients who experienced serious toxicity often received other drugs, in addition to the interacting drug cited, that competed with the statin through CYP3A4.

Finally, the interaction of the statins with the fibric acid lipid-lowering agents like gemfibrozil and fenofibrate is thought to have a pharmacodynamic rather than a pharmacokinetic basis. Although rhabdomyolysis has been reported most frequently with lovastatin–fibrate combinations, there have also been cases reported with each of the other marketed statins, except possibly cerivastatin. Studies have not found any fibrate-dependent alterations in statin concentrations, however.[28,58] Moreover, statin-induced myopathy is seen with hypothyroidism[59–61] or congenital or acquired myopathic conditions.[62,63] This drug–disease interaction likely represents a statin-related functional mitochondrial deficit in addition to an inherent tendency toward muscular disease.

Summary

Drug interactions commonly occur in patients taking multiple medications. Although there may be some differences in the potential for statin preparations to be involved in serious adverse drug reactions, in general, they have a proven record of safety and efficacy in large clinical studies. Nonetheless, concern is warranted when statins, particularly lovastatin and simvastatin, are used in multidrug regimens because of dose-dependent toxicity and their propensity toward marked elevations in concentration if taken with drugs that inhibit first-pass metabolism.

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References

1. Nies AS, Spielberg SP. Principles of therapeutics. In: Hardman JG, Limbird LE, Molinoff PB, Ruddon RW, Gilman AG, editors. *Goodman and Gilman's the pharmacological basis of therapeutics*. 9th ed. New York: McGraw-Hill; 1996. p. 43-62.
2. Roland M, Tozer TN. Interacting drugs. In: Roland M, Tozer TN, editors. *Clinical pharmacokinetics*. 2nd ed. Philadelphia: Lea and Febiger; 1989. p. 255-75.
3. Hansten PD. Understanding drug–drug interactions. *Science & Medicine* 1998;5(1):16-25.
4. Ross EM. Mechanisms of drug action and the relationship between drug concentration and effect. In: Hardman JG, Limbird LE, Molinoff PB, Ruddon RW, Gilman AG, editors. *Goodman and Gilman's the pharmacological basis of therapeutics*. 9th ed. New York: McGraw-Hill; 1996. p. 29-42.
5. Pounder DJ, Hartley AK, Watmough PJ. Postmortem redistribution and degradation of dothiepin. Human case studies and an animal model. *Am J Forensic Med Pathol* 1994;15:231-5.
6. Fromm MF, Kim RB, Stein CM, Wilkinson GR, Roden DM. Inhibition of P-glycoprotein-mediated drug transport: a unifying mechanism to explain the interaction between digoxin and quinidine. *Circulation* 1999;99:552-7.
7. Roland M, Tozer TN. Variability. In: Roland M, Tozer TN, editors. *Clinical pharmacokinetics*. 2nd ed. Philadelphia: Lea and Febiger; 1989. p. 197-212.
8. Herman RJ, Szakacs CBN, Verbeeck RK. Analysis of polymorphic variation in drug metabolism: II. Effects of data transformation on the sensitivity and specificity of modal detection. *Clin Invest Med* 1994;17:290-6.
9. Bertz RJ, Granneman GR. Use of in vitro and in vivo data to estimate the likelihood of metabolic pharmacokinetic interactions. *Clin Pharmacokinet* 1997;32:210-58.
10. Michalets EL. Update: clinically significant cytochrome P-450 drug interactions. *Pharmacotherapy* 1998;18:84-112.
11. Nelson DR, Koymans L, Kamataki T, Stegeman JJ, Feyereisen R, Waxman DJ, et al. P450 superfamily: update on new sequences, gene mapping, accession numbers and nomenclature. *Pharmacogenetics* 1996;6:1-42.
12. Rendic S, Di Carlo FJ. Human cytochrome P450 enzymes: a status report summarizing their reactions, substrates, inducers and inhibitors. *Drug Metab Rev* 1997;29:413-580.
13. Guengerich FP. Human cytochrome P450 enzymes. In: Ortiz de Montellano PR, editor. *Cytochrome P450 — structure, mechanism and biochemistry*. 2nd ed. New York: Plenum Press; 1995. p. 473-535.
14. Spatzeneger M, Jaeger W. Hepatic P450 in drug metabolism. *Drug Metab Rev* 1995;27:397-417.
15. Cupp MJ, Tracy TS. Cytochrome P450: new nomenclature and clinical implications. *Am Fam Physician* 1998;57:107-16.
16. Yue QY, Svensson JO, Alm C, Sjoqvist F, Sawe J. Codeine *o*-demethylation co-segregates with polymorphic debrisequine hydroxylation. *Br J Clin Pharmacol* 1989;28:639-45.
17. Ozdemir V, Fourie J, Busto U, Naranjo CA. Pharmacokinetic changes in the elderly. Do they contribute to drug abuse and dependence? *Clin Pharmacokinet* 1996;31:372-85.
18. Hunt CM, Westerkam WR, Stave GM. Effect of age and gender on the activity of human hepatic CYP3A. *Biochem Pharmacol* 1992;44:275-83.
19. Grundy SM. HMG–CoA reductase inhibitors for treatment of hypercholesterolemia. *N Engl J Med* 1988;319:24-33.
20. Tobert JA. Efficacy and long-term adverse effect pattern of lovastatin. *Am J Cardiol* 1988;62:28J-34J.
21. Pedersen TR, Berg K, Cook TJ, Faergeman O, Haghfelt T, Kjekshus J, et al. Safety and tolerability of cholesterol lowering with simvastatin during 5 years in the Scandinavian simvastatin survival study. *Arch Intern Med* 1996; 156:2085-92.
22. Hrab RV, Hartman HA, Cox RH Jr. Prevention of fluvastatin-induced toxicity, mortality, and cardiac myopathy in pregnant rats by mevalonic acid supplementation. *Teratology* 1994;50:19-26.
23. Flint OP, Masters BA, Gregg RE, Durham SK. HMG–CoA reductase inhibitor-induced myotoxicity: pravastatin and lovastatin inhibit the geranylgeranylation of low-molecular-weight proteins in neonatal rat muscle culture. *Toxicol Appl Pharmacol* 1997;145:99-110.
24. Gadbut AP, Caruso AP, Galper JB. Differential sensitivity of C2-C12 striated muscle cells to lovastatin and pravastatin. *J Mol Cell Cardiol* 1995;27:2397-402.
25. Smith PF, Eydelloth RS, Grossman SJ, Stubbs RJ, Schwartz MS, Germershausen JI, et al. HMG–CoA reductase inhibitor-induced myopathy in the rat: cyclosporine A interaction and mechanism studies. *J Pharmacol Exp Ther* 1991;257:1225-35.

26. Davidson MH, Stein EA, Dujovne CA, Hunninghake DB, Weiss SR, Knopp RH, et al. The efficacy and 6-week tolerability of simvastatin 80 and 160 mg/day. *Am J Cardiol* 1997;79:38-42.
27. Nakai A, Nishikata M, Uchida T, Ichikawa M, Matsuyama K. Enhanced myopathy following administration of hypolipidemic agents under urethane anesthesia. *Biol Pharm Bull* 1997;20:104-6.
28. Pierce LR, Wysowski DK, Gross TP. Myopathy and rhabdomyolysis associated with lovastatin-gemfibrozil combination therapy. *JAMA* 1990;264:71-5.
29. Abdul-Ghaffar NU, el-Sonbaty MR. Pancreatitis and rhabdomyolysis associated with lovastatin–gemfibrozil therapy. *J Gastroenterol* 1995;21:340-1.
30. Van Puijenbroek EP, Du Buf-Vereijken PWG, Spooren PFMJ, van Doormaal JJ. Possible increased risk of rhabdomyolysis during concomitant use of simvastatin and gemfibrozil. *J Intern Med* 1996;240:403-4.
31. Tal A, Rajeshawari M, Isley W. Rhabdomyolysis associated with simvastatin–gemfibrozil therapy. *South Med J* 1997;90:546-7.
32. Garnett WR. Interactions with hydroxymethylglutaryl–coenzyme A reductase inhibitors. *Am J Health Syst Pharm* 1995;52:1639-45.
33. Spach DH, Bauwens JE, Clark CD, Burke WG. Rhabdomyolysis associated with lovastatin and erythromycin use. *West J Med* 1991;154:213-5.
34. Lees RS, Lees AM. Rhabdomyolysis from the coadministration of lovastatin and the antifungal agent itraconazole. *N Engl J Med* 1995;333:664-5.
35. Horn M. Coadministration of itraconazole with hypolipidemic agents may induce rhabdomyolysis in healthy individuals [letter]. *Arch Dermatol* 1996; 132:1254.
36. Meier C, Stey C, Brack T, Maggiorini M, Risti B, Krahenbuhl S. Rhabdomyolysis in patients treated with simvastatin/ciclosporin: role of the hepatic P450 enzyme. *Schweiz Med Wochenschr* 1995;125:27-8.
37. Barclay P, O'Connell P. Clarithromycin drug interactions complicating cyclosporine and simvastatin therapy [abstract]. *Austral J Hosp Pharm* 1996; 26:180.
38. Ahmad S. Diltiazem myopathy [letter]. *Am Heart J* 1993;126:1494-5.
39. Lennernas H, Fager G. Pharmacodynamics and pharmacokinetics of the HMG–CoA reductase inhibitors. Similarities and differences. *Clin Pharmacokinetics* 1997;32:403-25.
40. Lea AP, McTavish D. Atorvastatin. A review of its pharmacology and therapeutic potential in the management of hyperlipidemias. *Drugs* 1997;53:828-47.
41. Boberg M, Angerbauer R, Fey P, Kanhai WK, Karl W, Kern A, et al. Metabolism of cerivastatin by human liver microsomes in vitro. Characterization of primary metabolic pathways and of cytochrome P450 enzymes involved. *Drug Metab Dispos* 1997;25:321-31.
42. Transon C, Leemann T, Vogt N, Dayer P. In vivo inhibition profile of cytochrome P450TB (CYP2C9) by (+/-)-fluvastatin. *Clin Pharmacol Ther* 1995;58:412-7.
43. Jacobsen W, Kirchner G, Hallensleben K, Mancinelli L, Deters M, Hackbarth I, et al. Comparison of cytochrome P450-dependent metabolism and drug interactions of the 3-hydroxy-3-methylglutaryl–CoA reductase inhibitors lovastatin and pravastatin in the liver. *Drug Metab Dispos* 1999;27:173-9.
44. Kocarek TA, Reddy AB. Regulation of cytochrome P450 expression by inhibitors of hydroxymethylglutaryl–coenzyme A reductase in primary cultured rat hepatocytes and in rat liver. *Drug Metab Dispos* 1996;24:1197-204.
45. Mauro VF. Clinical pharmacokinetics and practical applications of simvastatin. *Clin Pharmacokinet* 1993;24:195-202.
46. Muck W, Ritter W, Ochmann K, Unger S, Ahr G, Wingender W, et al. Absolute and relative bioavailability of the HMG–CoA reductase inhibitor cerivastatin. *Int J Clin Pharmacol Ther* 1997;35:255-60.
47. Azie E, Brater DC, Becker PA, Jones DR, Hall SD. The interaction of diltiazem with lovastatin and pravastatin. *Clin Pharmacol Ther* 1998;64:369-77.
48. Neuvonen PJ, Jalava KM. Itraconazole drastically increases plasma concentrations of lovastatin and lovastatin acid. *Clin Pharmacol Ther* 1996;60:54-61.
49. Kivisto KT, Kantola T, Neuvonen PJ. Different effects of itraconazole on the pharmacokinetics of fluvastatin and lovastatin. *Br J Clin Pharmacol* 1998;46:49-53.
50. Neuvonen PJ, Kantola T, Kivisto KT. Simvastatin but not pravastatin is very susceptible to interaction with the CYP3A4 inhibitor itraconazole. *Clin Pharmacol Ther* 1998;63:332-41.
51. Neuvonen PJ, Kantola T, Kivisto KT. Effect of itraconazole on the pharmacokinetics of atorvastatin. *Clin Pharmacol Ther* 1998;64:58-65.
52. Yang BB, Siedlik PH, Smithers JA, Sedman AJ, Stern RH. Atorvastatin pharmacokinetic interactions with other CYP3A4 substrates: erythromycin and ethinyl estradiol [abstract]. *Pharm Res* 1996;13:S437.
53. Muck W, Ochmann K, Rohde G, Unger S, Kuhlmann J. Influence of erythromycin pre- and co-treatment on single-dose pharmacokinetics of the HMG–CoA reductase inhibitor cerivastatin. *Eur J Clin Pharmacol* 1998;53:469-73.
54. Bradford RH, Shear CL, Chremos AN, Dujovne CA, Franklin FA, Grillo RB, et al. Expanded Clinical Evaluation of Lovastatin (EXCEL) study results: two-year efficacy and safety follow-up. *Am J Cardiol* 1994;74:667-73.
55. Haria M, McTavish D. Pravastatin: a reappraisal of its pharmacological properties and clinical effectiveness in the management of coronary heart disease. *Drugs* 1997;53:299-336.
56. Hsu I, Spinler SA, Johnson NE. Comparative evaluation of the safety and efficacy of HMG–CoA reductase inhibitor monotherapy in the treatment of primary hypercholesterolemia. *Ann Pharmacother* 1995;29:743-59.
57. Ballantyne CM, Bourge RC, Domalik LJ, Eisen HJ, Fishbein DP, Kubo SH, et al. Treatment of hyperlipidemia after heart transplantation and rationale for the heart transplant lipid registry. *J Am Coll Cardiol* 1996;78:532-5.
58. Spence JD, Munoz CE, Hendricks L, Latchinian L, Khouri HE. Pharmacokinetics of the combination of fluvastatin and gemfibrozil. *Am J Cardiol* 1995;76:80A-83A.
59. Scalvini T, Marocolo D, Cerudelli B, Sleiman I, Balestrieri GP, Giustina G. Pravastatin-associated myopathy. Report of a case. *Recenti Prog Med* 1995;86:198-200.
60. Galiana J, Marchan E, Montes I, Pato S. Toxic myopathies related to the administration of hypolipidemic agents: Are the drugs the only things responsible? *Rev Clin Esp* 1995;195:620-2.
61. Lang JE, Wang P, Glueck CJ. Myopathy associated with lipid lowering therapy in patients with previously undiagnosed or undertreated hypothyroidism. *Clin Chim Acta* 1996;254:65-92.
62. Hino I, Akama H, Furuya T, Ueda H, Taniguchi A, Hara M, et al. Pravastatin-induced rhabdomyolysis in a patient with mixed connective tissue disease. *Arthritis Rheum* 1996;39:1259-60.
63. Giordano N, Senesi M, Mattii G, Battisti E, Villanova M, Gennari C. Polymyositis associated with simvastatin. *Lancet* 1997;349(9065):1600-1.

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New insights into the pharmacodynamic and pharmacokinetic properties of statins

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Abstract

The beneficial effects of statins are assumed to result from their ability to reduce cholesterol biosynthesis. However, because mevalonic acid is the precursor not only of cholesterol, but also of many nonsteroidal isoprenoid compounds, inhibition of 3-hydroxy-3-methylglutaryl coenzyme A reductase may result in pleiotropic effects. It has been shown that several statins decrease smooth muscle cell migration and proliferation and that sera from fluvastatin-treated patients interfere with its proliferation. Cholesterol accumulation in macrophages can be inhibited by different statins, while both fluvastatin and simvastatin inhibit secretion of metalloproteinases by human monocyte-derived macrophages. The antiatherosclerotic effects of statins may be achieved by modifying hypercholesterolemia and the arterial wall environment as well. Although statins rarely have severe adverse effects, interactions with other drugs deserve attention. Simvastatin, lovastatin, cerivastatin, and atorvastatin are biotransformed in the liver primarily by cytochrome P450-3A4, and are susceptible to drug interactions when co-administered with potential inhibitors of this enzyme. Indeed, pharmacokinetic interactions (e.g., increased bioavailability), myositis, and rhabdomyolysis have been reported following concurrent use of simvastatin or lovastatin and cyclosporine A, mibefradil, or nefazodone. In contrast, fluvastatin (mainly metabolized by cytochrome P450-2C9) and pravastatin (eliminated by other metabolic routes) are less subject to this interaction. Nevertheless, a 5- to 23-fold increase in pravastatin bioavailability has been reported in the presence of cyclosporine A. In summary, statins may have direct effects on the arterial wall, which may contribute to their antiatherosclerotic actions. Furthermore, some statins may have lower adverse drug interaction potential than others, which is an important determinant of safety during long-term therapy.

Keywords: HMG-CoA reductase inhibitors; Pharmacodynamics; Pharmacokinetics; Statins

Abbreviations: AUC, area under the plasma concentration-time curve; CAD, coronary artery disease; CCYP, cytochrome P450; C_{max}, peak plasma concentration; eNOS, endothelial nitric oxide synthase; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; LDL, low-density lipoprotein; MMP, matrix metalloproteinase; MVA, mevalonic acid; SMC, smooth muscle cell; T_{max}, time to reach peak concentration.

Contents

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1. Introduction

The 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) can achieve relatively large reductions in plasma cholesterol levels (Brown & Goldstein, 1991; Havel & Rapaport, 1995) and represent an established class of drugs for the treatment of hypercholesterolemia. Several clinical trials have demonstrated that statins can ameliorate vascular atherosclerosis, and reduce cardiovascular-related morbidity and mortality, in patients with and without coronary artery disease (CAD) symptoms (Brown et al., 1993; Downs et al., 1998; Herd et al., 1997; Jukema et al., 1995; Riegger et al., 1999; Sacks et al., 1996; Scandinavian Simvastatin Survival Study Group, 1994; Shepherd et al., 1995; Long-Term Intervention with Pravastatin in Ischaemic Disease [LIPID] Study Group, 1998). These trials provide a powerful endorsement of the value of lipid-lowering therapy with a statin in patients who are at risk for CAD. Interestingly, there is now a variety of clinical and experimental evidence to show that some statins can interfere with major events involved in the formation of atherosclerotic lesions, independently of their hypolipidemic properties (Bellosta et al., 1998a; Corsini et al., 1998; Herd et al., 1997; O'Driscoll et al., 1997; Rosenson & Tangney, 1998; Williams et al., 1998). The contribution of these direct vascular effects to the cardiovascular event reduction observed in clinical trials of statins represents one of the major challenges for future studies in order to understand the antiatherosclerotic benefits of these agents.

Together with the identification of new pharmacologic targets, strategies to improve the therapeutic profile of statins will include optimization of safety and tolerability. Although statins rarely have severe adverse effects and are generally well tolerated, interactions with other drugs deserve attention (Garnett, 1995). Indeed, numerous factors contributing to the risk for adverse drug interactions with statins have been reported recently (Desager & Horsmans, 1996; Jokubaitis, 1994; Lennernas & Fager, 1997), and should be considered when patients are receiving additional drugs. Knowledge about the differences in the adverse interaction profile of statins is an important determinant of safety in long-term therapy of hypercholesterolemia.

The aim of this review is to discuss the current understanding of the pharmacodynamics and pharmacokinetics of statins. The mechanism(s) of the antiatherosclerotic action of statins that may contribute to the cardiovascular benefits observed in clinical trials and the available information regarding the relevant interactions occurring between statins and other classes of drugs are reviewed.

2. Direct antiatherosclerotic properties of statins: evidence for new pharmacologic targets

In patients with atherosclerosis, it has been assumed that any beneficial effects of statins are linked to their hypolipidemic properties (Feussner, 1994; Hunninghake, 1992; Kjekshus et al., 1996), thus suggesting that the hypolipidemic effect is the main mechanism for preventing the development of atherosclerosis. However, since mevalonic acid (MVA), the product of the effect of HMG-CoA reductase on HMG-CoA, is the precursor not only of cholesterol, but also of numerous metabolites (Goldstein & Brown, 1990; Grunler et al., 1994), inhibition of HMG-CoA reductase has the potential to result in pleiotropic effects (Bellosta et al., 1998a; Bernini et al., 1993; Corsini et al., 1993; Rosenson & Tangney, 1998). The beneficial effect of statins on clinical events, therefore, may involve nonlipid-related mechanisms that modify endothelial function, inflammatory responses, plaque stability, and thrombus formation (Rosenson & Tangney, 1998). Indeed, the mevalonate pathway yields a series of isoprenoids (Fig. 1) that are vital for diverse cellular functions. These isoprenoids include isopentenyl adenosine, present in some types of transfer RNA; dolichols, required for glycoprotein synthesis; and polyisoprenoid side chains of ubiquinone and heme A, involved in electron transport (Goldstein & Brown, 1990; Grunler et al., 1994). Several proteins post-translationally modified by the covalent attachment of MVA-derived isoprenoid groups, either farnesyl- or geranylgeranyl-pyrophosphate, have been identified (Glomset et al., 1990; Goldstein & Brown., 1990; Maltese, 1990). These proteins must be prenylated as a prerequisite for membrane association, which is required for their function (Glomset et al., 1990; Maltese, 1990). Members of this family are involved in a number of cellular processes, including cell signaling, cell differentiation and proliferation, myelination, cytoskeleton dynamics, and endocytotic/exocytotic transport (Table 1).

Recently, we have demonstrated that lipophilic statins (e.g., fluvastatin, simvastatin) decrease smooth muscle cell (SMC) migration and proliferation, an effect that is independent of their hypocholesterolemic properties (Bellosta et al., 1998a; Corsini et al., 1996b; Soma et al., 1993). The in vitro inhibition of cell growth induced by statins (70–90% decrease) can be almost completely prevented by the addition of mevalonate and partially (80%) by all-*trans* farnesol and geranylgeraniol (Fig. 2), confirming the specific role of isoprenoid metabolites in regulating this cellular event (Corsini et al., 1993; Raiteri et al., 1997). One possible mechanism by which statins affect cell growth could be via interference with signaling pathways that require prenylated proteins (Goldstein & Brown, 1990; Maltese, 1990; Glomset et al., 1990). Identification of some of these proteins, e.g., nuclear laminin B, *ras* proto-oncogene, Rho-related proteins, and the γ-subunit of heterotrimeric GTP-binding proteins, supports the link between the mevalonate pathway, signal transduction, and cell cycle progression (Table 1).

The inhibitory effects of statins on SMC migration and proliferation might provide an example for a general pharmacodynamic mechanism of this class of drugs, whose effectiveness has been shown in different in vitro cellular models of atherosclerosis, from macrophages to endothelial cells (Table 2). For example, fluvastatin and simvastatin

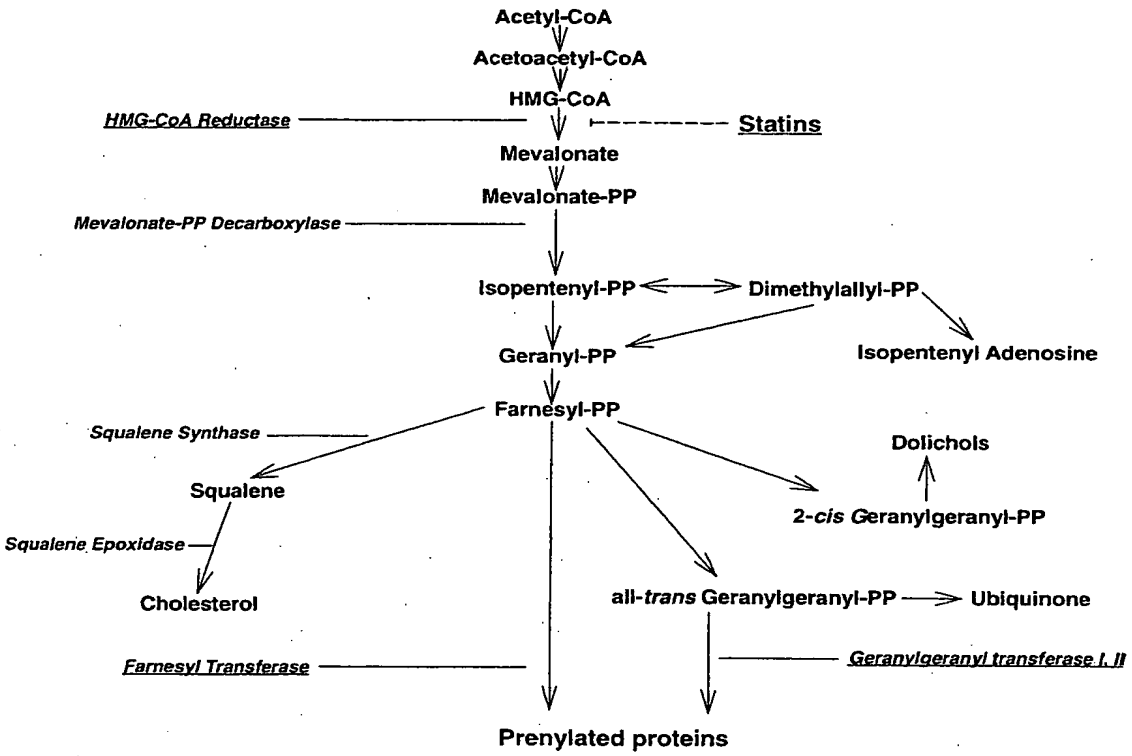

Fig. 1. The mammalian cell mevalonate pathway. PP, pyrophosphate.

may inhibit cholesterol accumulation in macrophages in vitro by inhibiting modified low-density lipoprotein (LDL) endocytosis (Bernini et al., 1995). Statins are also able to reduce the secretion of extracellular matrix-degrading enzyme (matrix metalloproteinase; MMP) by macrophages. These MMPs, once secreted and activated, can completely degrade all extracellular matrix components, weakening the fibrous cap of the atherosclerotic plaque. MMP activity, together with cholesterol accumulation by macrophages, may predispose the atherosclerotic lesion to fissuration (Dollery et al., 1995; Falk et al., 1995). We recently have shown that fluvastatin, at a concentration as low as 1 μM, inhibits the activity of gelatinase-B (MMP-9) in human monocyte-derived macrophages (Bellosta et al., 1998b). In addition, fluvastatin can inhibit MMP-9 secretion in a concentration-dependent manner; this inhibitory effect could be prevented by the simultaneous addition of MVA (Fig. 3). Similar results have been reported for simvastatin (Bellosta et al., 1998b), atorvastatin, and lovastatin (Bourcier et al., 1997). Interestingly, in all of these models, the activity of statins could be prevented or reversed by the addition of mevalonate and, in some cases, by farnesol or geranylgeraniol (Aminou et al., 1997; Baetta et al., 1997a, 1997b; Bernini et al., 1993; Corsini et al., 1993; Essig et al., 1998; Gonzalez & Badimon, 1996; Gonzalez et al., 1997; Hernandez-Perera et al., 1998; Laufs et al., 1998; Munro et al., 1994). Taking everything together, notwithstanding the limitations of in vitro studies, effects other than cholesterol reduction, but related to their primary mechanism of action (i.e., inhibition of HMG-CoA reductase), may potentially contribute to the antiatherosclerotic properties of statins (Bellosta et al., 1998a; Rosenson & Tangney, 1998).

These in vitro findings have been corroborated in rabbit models of atherogenesis, which demonstrated the ability of a variety of statins to reduce the extent of carotid and femoral intimal lesions induced by vascular injury (Bandoh et al., 1996; Igarashi et al., 1997; Soma et al., 1993, 1995b). In these in vivo models, the effect of statins was not associated with significant changes in serum cholesterol concentrations (Bandoh et al., 1996; Bocan et al., 1994; Gellman et al., 1991; Igarashi et al., 1997; Soma et al., 1993, 1995b; Zhu et al., 1992). Soma et al. (1993, 1995b) investigated the in vivo activity of statins on neointimal formation induced by insertion of a flexible collar around one carotid artery in normocholesterolemic rabbits. The various statins were administered to animals mixed with food at daily doses of 20 mg/kg for 2 weeks, starting from the day of collar insertion. All statins limited the increase of intimal thickening at this dose; fluvastatin was the most effective drug in this regard, followed by simvastatin, lovastatin, and pravastatin (in decreasing order). Reduction of neointimal hyperplasia ranged from about 50% for fluvastatin to about 10% for pravastatin, in the absence of changes in serum cholesterol concentration. Moreover, Soma et al. (1995b) demonstrated that lo-

Table 1
Role of prenylated proteins in cellular functioning

Molecular weight (kDa)	Comments
Farnesylated	
66–72	Nuclear laminin family
53–55	Unidentified proteins
41–46	Inositol triphosphate 5-phosphatase 2′, 3′-cyclic nucleotide 3′-phosphatase
37	Peroxisomal protein
21–28	Ras; involved in cell proliferation and differentiation
Geranylgeranylated[a]	
21–28	Rho/Rac/Cdc42; involved in cytoskeletal assembly, superoxide generation, cell cycle progression
	Rab; involved in transport of vesicles
	Rap; involved in cellular replication, platelet activation, generation of oxygen radicals
5–8	G-proteins (γ-subunit); involved in signal transduction

[a] In mammalian cells, 0.5–1% of total cellular proteins are geranylgeranylated. Data from Brown and Goldstein (1991), De Angelis and Braun (1996), De Smedt et al. (1996), Goldstein and Brown (1990), Glomset et al. (1990), Inglese et al. (1995), Laxminarayan et al. (1994), Maltese (1990), Tapon and Hall (1997), and Zhang and Casey (1996).

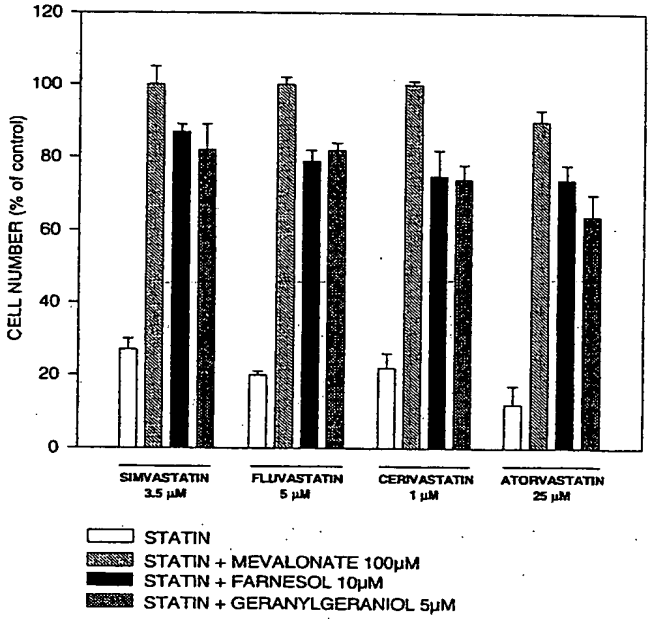

Fig. 2. Mevalonate and its derivatives prevent arterial smooth muscle cell growth inhibition by statins. Each bar represents the mean and SD of triplicate experiments. The mean (± SD) value of control (cells incubated with medium alone) was $1286 \times 10^3 \pm 41 \times 10^3$ cells/plate (taken as 100%). Duncan's test: all the treatments are statistically different from control $P < 0.01$, with the exception of simvastatin, fluvastatin, and cerivastatin plus mevalonate (Corsini et al., 1993; Raiteri et al., 1997).

cal arterial delivery of MVA at the site of collar placement fully prevented the inhibitory effect of fluvastatin (administered i.p. 5 mg/kg/day for 5 days) on neointimal hyperplasia induced by vascular injury, demonstrating that the mechanism underlying the antiproliferative effect is related to the local inhibition of mevalonate synthesis in SMC.

Several in vitro and preclinical observations support the hypothesis that apoptosis may also be involved in the modulation of the cellularity of the arterial wall in proliferative atherosclerotic or restenotic lesions where the SMC component is predominant (Bochaton Piallant et al., 1995; Han et al., 1995; Isner et al., 1995; Kockx et al., 1994). Indeed, statins have been reported to induce apoptosis in cultured vascular SMC (Soma et al., 1995a), and we have provided in vitro and in vivo evidence that statins may play an important role in cell cycle progression and apoptotic cell death in experimental models of SMC proliferation (Baetta et al., 1997a). In the latter study, the exposure of cultured SMC to statins (atorvastatin, fluvastatin, and simvastatin; 1–10 μM) induced a pronounced concentration-dependent decrease in cells within the S phase and an accumulation of cells in the G_2/M phases at 24 hr, particularly at the highest concentrations used. This resulted in an inhibitory effect on cell proliferation at 48 hr, followed by apoptosis when incubation was prolonged to 72 hr. These effects were fully prevented by mevalonate. The concentrations utilized in this study are not achievable in plasma of patients treated with regular doses of statin; thus, the clinical relevance of this study is questionable. Nevertheless, in the same study a pro-apoptotic effect of HMG-CoA reductase inhibitors was also demonstrated in vivo. Injured carotid arteries obtained from rabbits treated with either atorvastatin or fluvastatin (5 mg/kg/day starting 5 days prior to collar insertion) revealed an increased amount of apoptotic SMC, as compared with controls (Baetta et al., 1997a). Evidence from studies in a variety of tumor cell lines (Jones et al., 1994; Perez-Sala & Mollinedo, 1994; Perez-Sala et al., 1995) and experimental models of tumor growth (Baetta et al., 1997b; Soma et al., 1995a) confirms induction of apoptotic cell death in the presence of statins. Taken together, these results suggest that HMG-CoA reductase inhibitors may affect neointimal cellularity by modulating both SMC proliferation and apoptosis, probably via inhibition of isoprenoid biosynthesis and subsequent protein prenylation.

In addition, in vivo models demonstrate that statin therapy can stabilize atherosclerotic plaques and improve endothelial function in the absence of significant changes in serum cholesterol levels (Williams et al., 1998). The latter result is consistent with the finding that statins can directly up-regulate endothelial nitric oxide synthase (eNOS) expression in vitro under cholesterol-clamped conditions (Laufs et al., 1997, 1998; Liao et al., 1995; Kaesemeyer, 1999). Recently, Endres et al. (1998) reported that prophylactic treatment with statins augments cerebral blood flow, reduces cerebral infarct size, and improves neurological function in normocholesterolemic mice. This stroke protection was associated with up-regulation of eNOS without

Table 2
Potential mechanisms for a direct vascular action of statins

Lipid effects
Inhibition of cholesterol biosynthesis (Endo, 1992)
Increased uptake and degradation of LDL (Goldstein & Brown, 1990)
Inhibition of LDL oxidation (Aviram et al., 1992; Giroux et al., 1993; Hussein et al., 1997)
Inhibition of scavenger receptor expression (Umetani et al., 1996)
Inhibition of lipoprotein secretion (La Ville et al., 1984)
Inhibition of modified LDL endocytosis (Bernini et al., 1995)
Antiatherosclerotic effects
Inhibition of migration and proliferation of arterial myocytes (Corsini et al., 1993, 1996a, 1996b; Soma et al., 1993)
Inhibition of macrophage growth (Sakai et al., 1997)
Inhibition of cholesterol accumulation in macrophages (Bernini et al., 1993, 1995; Cignarella et al., 1998)
Inhibition of metalloproteinase secretion (Bellosta et al., 1998b)
Inhibition of cell adhesion (Masaaki et al., 1997)
Inhibition of tissue factor expression and activity (Colli et al., 1997)
Inhibition of superoxide generation (Giroux et al., 1993)
Inhibition of endothelin-1 synthesis and expression (Hernandez-Perera et al., 1998)
Increased expression and activity of eNOS (Endres et al., 1998; Kaesemeyer, 1999; Laufs et al., 1997, 1998)
Increased fibrinolytic activity (Essig et al., 1998)
Induction of myocyte apoptosis in proliferative lesions (Baetta et al., 1997a, 1997b; Guijarro et al., 1998)

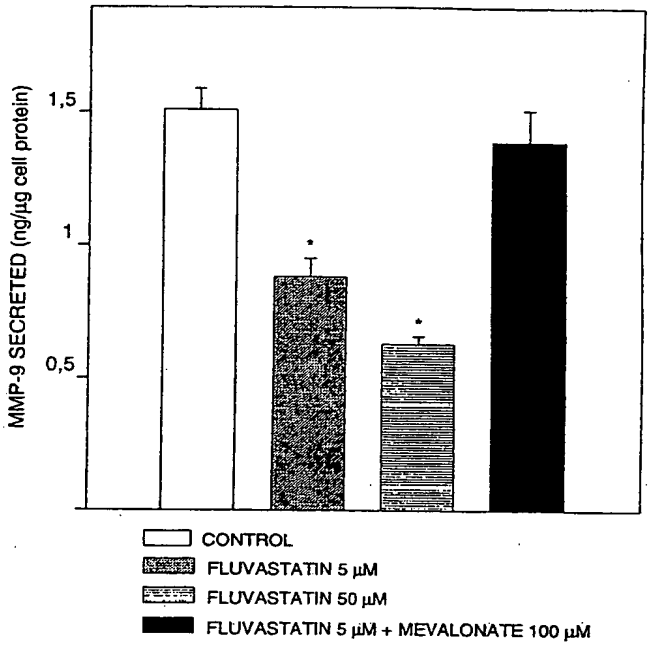

Fig. 3. Inhibitory effect of fluvastatin on the secretion of MMP-9 (gelatinase-B) by human macrophages in vitro. Each bar represents the mean and SD of two experiments performed in triplicate. Student's t test: $^*P < 0.01$ vs. control (Bellosta et al., 1998b).

changes in plasma cholesterol levels. Taken together, these studies suggest that statins can directly protect the arterial wall, irrespective of plasma lipid changes.

A further step towards validation of experimental evidence of direct antiatherosclerotic effects of statins is provided by an ex vivo study addressing the effect of sera from statin-treated patients on SMC proliferation (Corsini et al., 1996b, 1998). The ability of fluvastatin to inhibit SMC proliferation at therapeutically relevant concentrations (0.1–1 µM) (Dain et al., 1993; Tse et al., 1992) prompted us to investigate the pharmacologic activity of sera from patients treated with fluvastatin, as compared with the more hydrophilic pravastatin, which has a lipid-lowering effect similar to fluvastatin, but without in vitro effects on SMC proliferation. Fluvastatin and pravastatin, both administered at a dosage of 40 mg twice daily for 6 days, resulted in similar decreases in LDL-cholesterol in patients with Type IIa hypercholesterolemia. Cholesterol biosynthesis and cell proliferation in cultured human SMC exposed to 15% sera from these patients showed striking differences between the fluvastatin and pravastatin treatment groups. With pravastatin, essentially no changes in cholesterol biosynthesis or SMC proliferation were seen throughout 6 hr of exposure. In contrast, sera from fluvastatin-treated patients caused a 43% inhibition of cholesterol biosynthesis in SMC and a time-dependent reduction of cell proliferation (Corsini et al., 1996b). These findings are the first ex vivo demonstration of the antiproliferative activity of a HMG-CoA reductase inhibitor.

Recent clinical studies (Herd et al., 1997; O'Driscoll et al., 1997; Packard and West of Scotland Coronary Prevention Study Group, 1998) suggest that the antiatherosclerotic effects of these drugs indeed may extend beyond cholesterol reduction. Lipid-lowering clinical trials suggest that despite comparable reduction in serum cholesterol levels, the risk of cardiovascular events in statin-treated patients is lower compared with other agents or modalities used to decrease serum cholesterol levels (Buchwald et al., 1990). Furthermore, treatment with statins improves endothelial function in the absence of significant changes in serum cholesterol levels (O'Driscoll et al., 1997).

The nonlipid-related properties of statins may help to explain the early and significant cardiovascular event reduction reported in several clinical trials (Downs et al., 1998; Scandinavian Simvastatin Survival Study Group, 1994; Shepherd et al., 1995). Additional support for this hypothesis is provided by the Lipoprotein and Coronary Atherosclerosis Study (Herd et al., 1997), which investigated the progression and regression of atherosclerotic lesions after fluvastatin treatment. The results of this angiographic trial showed that treatment with fluvastatin reduces the progression of atherosclerotic lesions, as well as cardiovascular clinical endpoints. The beneficial effect was consistent across all baseline LDL-cholesterol subgroups from normal to moderately hypercholesterolemic patients.

In summary, experimental and clinical evidence suggests that statins can exert a direct antiatherosclerotic effect on the arterial wall, beyond their lipid-lowering properties, which could translate into a more significant prevention of cardiovascular disease.

3. Clinical pharmacokinetics of statins with reference to drug interactions

The issue of safety and drug tolerance is particularly important in primary and secondary prevention of cardiovascular disease, where the risks of long-term therapy must be considered in the context of achievable benefits. In general, statin monotherapy is well tolerated and has a low frequency of adverse events (Dujovne et al., 1991; Hsu et al., 1995). The most important adverse effects associated with statins are myopathy and asymptomatic increase in hepatic transaminases, which occur rarely (Christians et al., 1998; Hsu et al., 1995). However, since statins are prescribed on a long-term basis, possible interactions with other drugs deserve attention, as many patients will typically receive drugs for concomitant conditions during the course of statin therapy.

While the possibility for drug-drug interactions within the body is almost limitless, a drug interaction generally occurs when either the pharmacokinetics or pharmacodynamics of one drug is altered by another (Gibaldi, 1991; Stockley, 1996). Many drug-drug interactions have been demonstrated in clinical experience with statins (Christians et al., 1998; Garnett, 1995; Tobert, 1988), and in many respects, these interactions are qualitatively and quantitatively comparable for all agents of this class. Some of these interactions are indeed of minor clinical consequence because they neither compromise cholesterol-lowering activity nor increase toxicity. Due to differences in statin physicochemical and pharmacokinetic properties, however, some important differences in their interaction potential are evident. Of particular note are interactions with other lipid-lowering agents, such as fibrates and niacin (nicotinic acid), immunosuppressive agents, macrolides, and antifungal imidazoles, which may lead to severe skeletal muscle toxicity and rhabdomyolysis (Garnett, 1995; Tobert, 1988). It is important to mention that myopathy occurring during therapy with statins is usually the result of a complex interaction between drug, disease, and concomitant therapy (Bays & Dujovne, 1998; Christians et al., 1998). The adverse effects that occur when statins are co-administered with other drugs usually correlate with increased systemic concentrations of the HMG-CoA reductase inhibitor (Garnett, 1995). While it is well known that plasma concentrations of statins (with the exception of atorvastatin) (Cilla et al., 1996) do not correlate with their lipid-lowering activity, increased plasma drug levels have been regarded as an index of potential untoward effects in peripheral tissues (Hamelin & Turgeon, 1998).

Overall, the statins are very selective inhibitors of HMG-CoA reductase and do not show any relevant affinity towards other enzymes or receptor systems (Corsini et al., 1995). Therefore, at the pharmacodynamic level, statins are not prone to interference with other drugs. At the pharmacokinetic level, however, interactions can affect the processes by which statins are absorbed, distributed, metabolized, and excreted. The following section summarizes the pharmacokinetic profiles of statins and examines some of the specific drug-drug interactions that have been reported to date (Table 3).

Table 3
Summary of clinically relevant drug interactions with statins reported in the literature

Drug	Fluvastatin	Lovastatin	Pravastatin	Simvastatin
Warfarin	+	+	−	+
Digoxin	2	2	2	1
Niacin (nicotinic acid)	2	1	2	2
Erythromycin	2	1	2	2
Cyclosporine A	2	1	1	1
Fibrates	2	1	1	1

+, interaction reported; −, no interaction reported.

3.1. Pharmacokinetics

Comparative pharmacokinetic data among the clinically available statins have been provided by several authors (Appel & Dingemanse, 1996; Christians et al., 1998; Desager & Horsmans, 1996; Lennernas & Fager, 1997), and are summarized in Table 4. With the exception of lovastatin and simvastatin (which are administered as lactone prodrugs and must be hydrolyzed in vivo to the corresponding β-hydroxy acid to achieve pharmacologic activity) (Corsini et al., 1995), all statins are administered as the active β-hydroxy acid form.

3.1.1. Absorption

The extent of absorption of HMG-CoA reductase inhibitors varies considerably from 30% to 98% (Appel & Dingemanse, 1996; Christians et al., 1998; Dain et al., 1993; Mück, 1998; Mück et al., 1999, 1997a, 1997c; Posvar et al., 1996; Tse et al., 1992). All statins are absorbed rapidly following oral administration, with time to reach peak concentrations (T_{max}) of within 4 hr. As a result of saturable first-pass metabolism (Dain et al., 1993; Tse et al., 1992), fluvastatin is the only statin able to achieve peak plasma concentrations (C_{max}) in the micromolar range, which could potentially have clinical relevance for explaining its direct antiatherosclerotic activity (e.g., inhibition of SMC proliferation). With the shortest elimination half-life of available statins and inactive metabolites (see Table 4), however, systemic exposure of fluvastatin is relatively short-lived.

Food intake has a variable effect on the absorption of statins. While no effect is apparent for simvastatin and cerivastatin, plasma levels of lovastatin are increased and bioavailability of fluvastatin, pravastatin, and atorvastatin is decreased. The overall hypocholesterolemic efficacy of statins, however, is not affected by whether the statin is taken with the evening meal or at bedtime (Garnett, 1995). With the exception of cerivastatin (Mück et al., 1997c), all statins posses a low systemic bioavailability, indicating extensive first-pass extraction.

In general, drug interactions can occur at different levels of absorption, such as complex formation, changes in gastrointestinal pH and motility, and competitive transport by carriers in the intestinal mucosa (Gibaldi, 1991; Stockley, 1996). Concomitant administration of bile acid sequestrants (e.g.,

Table 4
Comparative clinical pharmacokinetics of statins

Parameter	Atorvastatin	Cerivastatin	Fluvastatin	Lovastatin	Pravastatin	Simvastatin
Absorption						
Fraction absorbed (%)	30	98	98	30	34	60–80
T_{max} (hr)	2–3	2.5	0.5–1	2–4	0.9–1.6	1.3–2.4
C_{max} (ng/mL)	27–66	2	448	10–20	45–55	10–34
Bioavailability (%)	12	60	19–29	5	18	5
Effect of food	↓13%	0	↓15% to ↑25%	↑50%	↓30%	0
Distribution						
Fraction bound (%)	80–90	>99	>99	>95	43–55	94–98
Lipophilicity, $C \log P$ (octanol/water)	4.06 (1,482)	1.47 (29.51)	3.24 (1738)	4.27 (18,620)	−0.22 (0.60)	4.68 (47,860)
Metabolism						
Hepatic extraction (%)	>70	NA	>68	>70	46–66	78–87
Systemic metabolites	Active	Active	Inactive	Active	Inactive	Active
Clearance (L/hr/kg)	0.25	0.20	0.97	0.26–1.1	0.81	0.45
Excretion						
$t_{1/2}$ (hr)	15–30	2.1–3.1	0.5–2.3	2.9	1.3–2.8	2–3
Urinary excretion (%)	Negligible	30	6	10	20	13
Fecal excretion (%)	Major route	70	90	83	71	58

Based on a 40 mg oral dose, with the exception of cerivastatin (0.2 mg). NA, no available data at present; $t_{1/2}$, terminal elimination half-life.

cholestyramine or colestipol) and pravastatin or fluvastatin results in an approximate 50% decrease in the area under the plasma concentration-time curve (AUC) for both statins (Appel & Dingemanse, 1996; Pan et al., 1990; Smith et al., 1993), without affecting the clinical efficacy of the combined therapy. Bile acid sequestrants also have been shown to reduce lovastatin intestinal absorption (Henwood & Heel, 1995). For cerivastatin, concomitant administration of cholestyramine decreases relative bioavailability by 21%, delays absorption (as evinced by an increased T_{max}), and reduces C_{max} by 41% (Mück et al., 1997b). This pharmacokinetic interaction can be minimized by the administration of cholestyramine 1 hr before meals, with cerivastatin administered either with the evening meal or at bedtime (Mück et al., 1997b).

It is important to mention a small study showing that fibers (pectin and oat bran) can reduce the cholesterol-lowering efficacy of lovastatin (Richter et al., 1991), presumably via reduced drug absorption. Indeed, when pectin and oat bran were stopped, plasma cholesterol levels decreased again in the presence of lovastatin therapy. Separating their administration can minimize the effects of this interaction. To date, however, no studies detailing the effect of fibers on the therapeutic efficacy of other statins have been published.

The effects of changes in gastrointestinal pH caused by the antacid Maalox® (magnesium hydroxide/aluminium hydroxide) on the pharmacokinetics of cerivastatin have been investigated recently (Mück et al., 1997a). The results of this study clearly demonstrate the lack of effect of changes in gastrointestinal pH on the bioavailability of cerivastatin. Similarly, concomitant administration of cimetidine did not affect cerivastatin pharmacokinetics (Mück et al., 1997a). The lack of influence of Maalox® and cimetidine on the pharmacokinetics of pravastatin has also been reported (Garnett, 1995; Stockley, 1996). In contrast, concomitant administration of fluvastatin and cimetidine, ranitidine, or omeprazole results in significant increases in fluvastatin C_{max} (43%, 70%, and 50%, respectively) and AUC (24–33%), while plasma clearance is decreased by 18–23% (Jokubaitis, 1994). These changes in fluvastatin pharmacokinetics may be due to several mechanisms, such as reduced loss of fluvastatin through acid-catalyzed dehydration of the molecule's side chain or an inhibitory effect of these drugs on the mixed-function oxidase system. Finally, the co-administration of cimetidine does not alter either the rate or extent of atorvastatin absorption or its effect on LDL-cholesterol level (Stern et al., 1998).

3.1.2. Distribution

With the exception of pravastatin, all statins are highly bound to plasma proteins, of which albumin is the most important (Appel & Dingemanse, 1996; Christians et al., 1998; Lennernas & Fager, 1997; Mück, 1998; Posvar et al., 1996; Tobert, 1988). As a result of extensive protein binding, the extent of systemic exposure to unbound, pharmacologically active, drug, therefore, remains extremely low. Moreover, statins are highly extracted by the liver, and drug displacement interactions are of limited importance (Appel & Dingemanse, 1996; Desager & Horsmans, 1996; Lennernas & Fager, 1997). Drug-drug interactions based on displacement of drugs highly bound to plasma proteins have not been demonstrated for fluvastatin, which shows the highest protein binding of all statins (Tse et al., 1993). Although circulating levels of unbound pravastatin are 10-fold higher than those of other statins as a result of low plasma protein binding (Quion & Jones, 1994), widespread tissue distribution is prevented by the hydrophilic nature of the drug (Hamelin & Turgeon, 1998). In addition, statins have a slow rate of onset of effect (Tobert, 1988) and are, therefore, insensitive to temporary changes in unbound plasma drug concentration.

3.1.3. Metabolism

After absorption, the liver biotransforms all statins, causing their low systemic bioavailability. The apparent total body clearance of all statins (Appel & Dingemanse, 1996; Desager & Horsmans, 1996; Lennernas & Fager, 1997; Mück, 1998; Mück et al., 1997c) is very high due to an important hepatic first-pass effect (Hamelin & Turgeon, 1998). Pravastatin is transformed enzymatically in liver cytosol (as well as nonenzymatically under acidic conditions before absorption in the gastric tract) into two relatively inactive metabolites (Kitazawa et al., 1993; Quion & Jones, 1994). In hepatocytes, the lactone forms of lovastatin and simvastatin undergo extensive metabolism by two distinct routes; namely, hydrolysis of the lactone ring to yield the active open form (Duggan & Vickers, 1990) and re-oxidation to the fused ring and further oxidation by the cytochrome P450 (CYP) 3A4 isoenzyme (Cheng et al., 1992; Wang et al., 1991). Cerivastatin (Mück, 1998) and atorvastatin (Stern et al., 1998; Yang et al., 1996) are also primarily metabolized by CYP3A4 to several active metabolites, while fluvastatin is predominantly (50–80%) (Fischer et al., 1999) metabolized by CYP2C9 to inactive metabolites (Appel & Dingemanse, 1996; Fischer et al., 1999; Transon et al., 1995). Recently, it has been shown that CYP3A4 and CYP2C8 also contribute to the metabolism of fluvastatin, albeit to a lesser extent (Fischer et al., 1999). Similarly, cerivastatin can also be biotransformed by CYP2C8 (Mück, 1998). The ability of fluvastatin and cerivastatin to be metabolized by multiple CYP isoenzymes, therefore, may avoid drug accumulation when one of these pathways is inhibited by co-administered drugs. Taken together, these results clearly show that statins, with the exception of pravastatin, are primarily biotransformed by the microsomal CYP isoenzyme systems.

Many drug-drug interactions are the result of inhibition or induction of CYP isoenzymes, which are responsible for the metabolism of more than 50% of the drugs currently available in clinical practice (Bertz & Granneman, 1997; Wrighton et al., 1996). Competition between drugs at the enzymatic level is common (Gibaldi, 1991; Stockley, 1996). This may serve to alter the disposition of statins, leading to increased plasma levels and greater risk of adverse events. Most of these interactions have been attributed to the inhibition of CYP3A4, one of the most important CYP isoforms responsible for drug metabolism (Thummel & Wilkinson, 1998). CYP3A4 is also the major enzyme metabolizing many statins, including lovastatin, simvastatin, cerivastatin, and atorvastatin (Table 5). Indeed, it has been reported that a pharmacokinetic interaction (e.g., increased bioavailability and cases of myositis and rhabdomyolysis) is associated with concurrent use of lovastatin or simvastatin and potential inhibitors of this enzyme, such as azole antifungals (ketoconazole, itraconazole) (Kivistö et al., 1998; Lees & Lees, 1995; Neuvonen et al., 1998), some macrolide antibiotics, including erythromycin (Ayanian et al., 1988; Garnett, 1995), and antidepressants (nefazodone) (Jacobson et al., 1997).

Of particular note is the interaction of simvastatin or lovastatin with the calcium-channel antagonist mibefradil, which recently was withdrawn from worldwide sale in the light of drug-interaction concerns (Anon, 1998). Mibefradil is metabolized by two hepatic pathways: hydrolysis of the ester bond in the side chain and oxidation by CYP3A4 (Ernst & Kelly, 1998). Metabolism by the oxidative pathway is inhibited after long-term administration of mibefradil, thus leading to possible interactions with other agents metabolized through the same pathway (Table 5). Indeed, 19 cases of simvastatin-associated rhabdomyolysis and 1

Table 5
Human CYP isoenzymes known to oxidize clinically used drugs

CYP1A2	CYP2C9	CYP2C19	CYP2D6	CYP2E1	CYP3A4
Acetaminophen	Alprenolol	Diazepam	Amitriptyline	Chloroxazone	**Atorvastatin**
Caffeine	Diclofenac	Ibuprofen	Bufaralol	Ethanol	**Cerivastatin**
Clozapine	**Fluvastatin**	Mephenytoin	Codeine	Halothane	Cyclosporine A
Phenacetin	*N*-Desmethyldiazepam	Methylphenobarbital	Debrisoquine	Paracetamol	Erythromycin
Theophylline	Tolbutamide	Omeprazole	Desipramine		Felodipine
	Warfarin	Proguanil	Dextromethorphan		Lidocaine
		Phenytoin	Encainide		**Lovastatin**
			Flecainide		Mibefradil
			Impramine		Midazolam
			Metoprolol		Nefazodone
			Mibefradil		Nifedipine
			Nortriptyline		Quinidine
			Perhexiline		**Simvastatin**
			Perphenazine		Triazolam
			Propafenone		Verapamil
			Propranolol		Warfarin
			Sparteine		
			Thioridazine		
			Timolol		

Modified from Brouwer et al. (1994).

case with lovastatin have been reported in patients receiving mibefradil (Schmassmann-Suhijar et al., 1998). Of the 19 patients given simvastatin, 9 also received cyclosporine A, which is known to increase circulating levels of this statin (Christians et al., 1998; Garnett, 1995). Indeed, plasma concentrations of simvastatin and its active metabolite (β-hydroxy-simvastatin) were increased as a result of the inhibition of their metabolism by mibefradil. In vitro interaction experiments in human liver microsomes confirm the ability of mibefradil to inhibit simvastatin and lovastatin metabolism, whereas the metabolism of pravastatin and fluvastatin is unaffected (Ernst & Kelly, 1998; Schmassmann-Suhijar et al., 1998). Mibefradil and its metabolite inhibit not only CYP3A4, but also CYP1A2 and CYP2D6 (Ernst & Kelly, 1998). Therefore, increased plasma concentrations of drugs metabolized by these isoenzymes could be expected when co-administered with mibefradil. Indeed, the active metabolites of simvastatin are biotransformed by CYP2D6 (Nordin et al., 1997), which could have potentiated the interaction with mibefradil.

Another interesting interaction occurs between statins and coumarin anticoagulants. Although the administration of lovastatin to patients receiving warfarin had no effect on prothrombin time, bleeding and/or increased anticoagulant effects have been reported in several patients taking anticoagulants concomitantly with lovastatin (Ahmad, 1990; Garnett, 1995; Hoffman, 1992). Simvastatin normally causes only a small, clinically irrelevant increase of the anticoagulant effects of warfarin (Walker, 1989). Nevertheless, a more marked anticoagulant effect and bruising have been seen in one patient (Gaw & Wosornu, 1992). Potential interaction between fluvastatin and warfarin has also been reported in some patients (Kline & Harrell, 1997; Trilli et al., 1996). Although none of the latter patients experienced clinically significant bleeding episodes, a reduction of warfarin dosage to achieve an appropriate level of anticoagulation was required. The mechanism of the interaction between statins and warfarin is uncertain. Warfarin is a racemic compound, consisting of a more potent (*S*)-enantiomer and a less potent (*R*)-enantiomer, although full anticoagulation still can be produced (Hirsh, 1991). The enantiomers are differentially metabolized by the CYP system in the liver: metabolism of (*R*)-warfarin is primarily catalyzed by CYP3A4 and CYP1A2 (Wilkinson, 1996), while (*S*)-warfarin is primarily metabolized by CYP2C9 (Rettie et al., 1992). Given that both CYP3A4 and CYP2C9 isoenzymes are involved in the metabolism of warfarin, competition with statins at this level may be a contributing factor in the observed interactions. However, the anticoagulant effects of warfarin are not known to be altered by co-administration of pravastatin (Quion & Jones, 1994), cerivastatin (Mück, 1998), or atorvastatin (Stern et al., 1997).

Advances in our understanding of the P-glycoprotein system further complicate an assessment of the interaction potential of HMG-CoA reductase inhibitors. Until recently, poor oral bioavailability of drugs was mainly attributed to low solubility in gastrointestinal fluids, low permeability through the mucosal membrane, and/or extensive hepatic first-pass metabolism. Drug interactions involving CYP isoenzymes were assumed to take place mainly in the liver (Christians et al., 1998). Recently, however, it has been recognized that CYP3A-mediated drug metabolism in the intestinal wall, together with P-glycoprotein counter-transport processes, significantly contribute to poor oral availability and play an important role in drug interactions (Christians et al., 1998). P-glycoproteins are transmembrane proteins that function as drug efflux pumps, and are capable of the active transport of drugs from intestinal, renal, and hepatic cells (Bertz & Granneman, 1997; Wacher et al., 1995). Originally recognized as one of the factors in multidrug resistance of various tumor cell lines, P-glycoprotein has received increasing attention as a significant factor in the elimination of a number of drugs that are CYP3A substrates (Bertz & Granneman, 1997). For example, P-glycoproteins are, at least in part, responsible for the low and variable oral availability of cyclosporine A, and both lovastatin and pravastatin are known P-glycoprotein substrates (Christians et al., 1998). The small intestine is, therefore, a potential site for drug interactions between this immunosuppressant and HMG-CoA reductase inhibitors, and this observation should be taken into account in managing hypercholesterolemic patients receiving cyclosporine A-based immunosuppressive therapy. Elevated LDL-cholesterol, for example, is often present in cardiac and renal transplant patients treated with cyclosporine A (Christians et al., 1998; Horl et al., 1989; Kasiske & Umen, 1987; Ong-Ajyooth et al., 1996), and cardiovascular complications are the most frequent cause of death in this group of patients (Aker et al., 1998; Braun & Marwick, 1994). Statins have been shown to effectively lower cholesterol levels in transplant patients receiving long-term cyclosporine A (Arnadottir et al., 1993, 1994; Holdaas et al., 1995; Kandus et al., 1998; Vanhaecke et al., 1994; Yoshimura et al., 1992), and are considered to be the most effective lipid-lowering drugs for use in such patients (Christians et al., 1998). The use of HMG-CoA reductase inhibitors in transplant patients, however, is complicated by the fact that there is an increased incidence of toxicity when these drugs are given in combination with cyclosporine A. Indeed, concomitant therapy with these agents has been reported to greatly increase the risk of myopathy that may eventually progress to rhabdomyolysis (Christians et al., 1998; Garnett, 1995; Goldberg & Roth, 1996; Olbricht et al., 1997; Tobert, 1988). Lovastatin, for example, has been associated with myopathy and rhabdomyolysis in cyclosporine A-treated transplant patients (Corpier et al., 1988; East et al., 1988). One source cites an incidence of myopathy of 0.15% for lovastatin monotherapy, increasing to 2%, 5%, and 28%, respectively, in patients receiving concomitant niacin, cyclosporine A plus niacin, and cyclosporine A plus gemfibrozil (Tobert, 1988). Concerns about rhabdomyolysis, therefore, are increased in transplant recipients because cyclosporine A is known to interact with cerivastatin, lovastatin, pravastatin, and simvastatin, increasing their plasma

levels (Table 6). In the light of these concerns, it has been recommended that statin therapy in cyclosporine A-treated transplant recipients is initiated at the lower end of the dosage range and titrated carefully (Christians et al., 1998). Although the mechanism of the cyclosporine A–statin interaction has not been completely elucidated, available evidence suggests that this interaction can occur at two different levels. First, lovastatin and simvastatin are substrates of the same CYP3A4 isoform as cyclosporine A (Christians et al., 1998; Wilkinson, 1996); competitive inhibition of this isoenzyme by cyclosporine A, therefore, may account for increased plasma levels of the statins (Arnadottir et al., 1993; Olbricht et al., 1997). The lack of an effect of lovastatin or simvastatin on cyclosporine A pharmacokinetics could be explained by the more than 10-fold higher concentrations of cyclosporine A and by a higher affinity of cyclosporine A to CYP3A4 (Combalbert et al., 1989). More recently, a 3- to 5-fold increase in plasma levels of another statin recognized by CYP3A4, cerivastatin, has been observed in patients receiving cyclosporine A (Mück, 1998). In contrast, fluvastatin shows a far milder interaction with cyclosporine A, since it is mainly recognized by CYP2C9 (Fischer et al., 1999); in fact, its bioavailability is increased less than 2-fold in the presence of cyclosporine A (Table 6). These findings give a clinical ground to the ongoing Assessment of Lescol® in Renal Transplantation trial, a multicenter, randomized study addressing the effect of fluvastatin on the incidence of cardiac event-free survival time in 2000 renal transplant patients (Holdaas et al., 1998).

The second mechanism that may explain the cyclosporine A–statin interaction is that these drugs are excreted predominantly in the bile, which may be affected by cyclosporine A-induced cholestasis (Klintmalm et al., 1981). Indeed, coadministration of cyclosporine A appears to alter the disposition of lovastatin, simvastatin, and pravastatin by decreasing biliary clearance, which in turn increases statin levels in the blood and skeletal muscle tissue in a rat model (Smith et al., 1991). In contrast to lovastatin, cerivastatin, and simvastatin, pravastatin is metabolized by nonCYP-dependent processes (Kitazawa et al., 1993). It can be speculated, therefore, that the interaction between cyclosporine A and pravastatin is at the level of pravastatin transport processes. Although it is known that pravastatin has a high affinity for a sodium-independent bile acid carrier system in the hepatocyte (Haria & McTavish, 1997), it remains to be demonstrated whether cyclosporine A interferes with this specific carrier system.

Additional interactions between statins and other drugs related to CYP-mediated metabolism, some of which are clinically relevant, have been reported. Inducers of CYP isoenzyme activity such as rifampicin, or inhibitors such as cimetidine, grapefruit juice, and verapamil, have been demonstrated to decrease or increase, respectively, pharmacokinetic parameters of some HMG-CoA reductase inhibitors (Garnett, 1995; Kantola et al., 1998a,b; Smith et al., 1993).

3.1.4. Excretion

The amount of the administered dose of statin that is excreted in urine varies from negligible amounts for atorvastatin (Posvar et al., 1996) to 20% and 30%, respectively, for pravastatin and cerivastatin (McClellan et al., 1998; Singhvi et al., 1990). In particular, pravastatin differs from other statins in that it shows a dual route of elimination. This is particular true when the drug is given intravenously; after this route of administration, the renal excretion of pravastatin attains 47% of the administered dose, corresponding to a renal clearance of about 450 mL/min (Lennernas & Fager, 1997). Tubular secretion is a predominant mechanism in the renal excretion of pravastatin (Halstenson et al., 1992). All other statins and their metabolites are excreted mainly via the bile into feces; insignificant amounts are excreted as parent drug (Appel & Dingemanse, 1996; Desager & Horsmans, 1996; Lennernas & Fager, 1997).

With the exception of atorvastatin (Posvar et al., 1996; Yang et al., 1996), the elimination half-life of all statins is very short (0.5–3 hr), and no drug accumulates in plasma on repeated administration (Appel & Dingemanse, 1996; Christians et al., 1998; Desager & Horsmans, 1996; Lennernas & Fager, 1997; Mück, 1998). It must be stressed that the reported pharmacokinetic half-lives of statins do not correspond with the duration of their pharmacodynamic effect (approximately 24 hr). However, due to its long elimination half-life and the presence of detectable plasma levels of active metabolites for a prolonged period of time (>24 hr) (Posvar et al., 1996), atorvastatin can accumulate in the plasma, achieving a steady-state drug concentration after multiple doses (Cilla et al., 1996). A correlation between the reduction in total cholesterol and LDL-cholesterol and the drug's pharmacokinetic profile has indeed been described for atorvastatin (Cilla et al., 1996).

Drug interactions at the excretion level might potentially occur as a consequence of competition for carrier-mediated transport across the bile canalicular membrane. As men-

Table 6
Summary of the pharmacokinetics of statins when co-administered with cyclosporine A

Parameter	Cerivastatin	Fluvastatin	Lovastatin	Pravastatin	Simvastatin
AUC (μg · hr/L)	↑3.7	↑1.9	↑20	↑5–23	↑3
C_{max} (μg/L)	↑4.8	↑1.3	—	↑8	—

Values shown are the changes relative to the statin alone.
Data from Arnadottir et al. (1993), Goldberg and Roth (1996), Mai et al. (1997), Olbricht et al. (1997), and Regazzi et al. (1993).

tioned in Section 3.1.3, inhibition of biliary excretion has been proposed as one possible mechanism for the interaction between cyclosporine A and statins (Smith et al., 1991).

3.1.5. Other interactions

Other drug interactions with statins have been reported, although the mechanisms responsible currently are unknown. Among these, interaction with fibric acid derivatives (fibrates) deserves particular attention because myopathy can occur with either drug alone and their effects, therefore, may be additive (Duell et al., 1998; Garnett, 1995; Miller & Spence, 1998; Pierce et al., 1990; Shepherd, 1995). Fibrates may impair liver function, which may result in a diminished hepatic extraction of statins, leading to higher plasma drug levels. Mild renal impairment in patients with myopathy may further increase the likelihood of rhabdomyolysis because fibrates are primarily renally excreted (Miller & Spence, 1998). However, it is important to note that plasma levels of active metabolites of lovastatin usually are not elevated in patients receiving gemfibrozil (Pierce et al., 1990), thus suggesting that this interaction may be pharmacodynamic in nature rather than pharmacokinetic. This contrasts with the situation in cases of lovastatin-associated rhabdomyolysis involving cyclosporine A (Corpier et al., 1988; East et al., 1988) and erythromycin (Ayanian et al., 1988; Garnett, 1995; Spach et al., 1991), in which levels of the statin were found to be grossly elevated.

Reports of myopathic complications have been published with the use of other statin–fibrate combinations, including pravastatin/gemfibrozil (Shepherd, 1995), simvastatin/gemfibrozil (Tal et al., 1997), cerivastatin/gemfibrozil (Pogson et al., 1999), atorvastatin/gemfibrozil (Duell et al., 1998), and pravastatin/clofibrate (Garnett, 1995). No differences in the pharmacokinetics of fluvastatin or gemfibrozil have been reported when these agents were co-administered (Garnett, 1995; Spence et al., 1995). In a review of all reported clinical trials involving statin-fibrate therapy, Shepherd (1995) estimated the rate of complications at 1%.

Myopathy has been reported in 2% of patients receiving lovastatin in combination with niacin (Garnett, 1995; Norman et al., 1988; Reaven & Witztum, 1988; Tobert, 1988). The mechanism behind this interaction is not completely elucidated. One plausible theory, which is consistent with cholesterol lowering, is that the interaction involves sarcolemmic membrane destabilization and increased membrane fluidity as a result of membrane cholesterol depletion (London et al., 1991; Pierce et al., 1990). However, plasma concentrations of the active metabolites of lovastatin were not elevated in patients receiving niacin. In addition, niacin monotherapy has not been reported to cause adverse musculoskeletal effects. It, therefore, remains unclear as to why niacin should precipitate myopathy in lovastatin-treated patients. No interactions have been documented when niacin was administered with simvastatin or pravastatin (Garnett, 1995; Hsu et al., 1995). Similarly, the concomitant administration of fluvastatin with niacin had no effect on the bioavailability of either drug (Smith et al., 1993).

Clinically important interactions have not been observed between statins and other drugs used in cardiovascular diseases, such as propranolol, calcium-channel antagonists (with the exception on mibefradil), angiotensin-converting enzyme inhibitors, and thiazide diuretics (Garnett, 1995; Pan et al., 1991; Peters et al., 1993; Smith et al., 1993). Although neither lovastatin nor pravastatin affects plasma digoxin concentrations, digoxin does increase the AUC of pravastatin (Garnett, 1995). Fluvastatin also has no effect on digoxin AUC or T_{max}, but small, clinically insignificant, increases in C_{max} and urinary clearance are apparent (Desager & Horsmans, 1996; Garnett et al., 1994). The only likely clinically relevant interaction between statins and digoxin is for simvastatin, which caused a slight elevation in plasma digoxin concentrations, leading to an increased "cardiac activity" in one study (Walker, 1989).

4. Conclusions

HMG-CoA reductase inhibitors, beyond their lipid-lowering properties, exert a direct antiatherosclerotic effect on the arterial wall that could prevent significant cardiovascular disease. Clinical trials have demonstrated that statins greatly reduce cardiovascular-related morbidity and mortality in patients with and without symptoms of CAD. Several issues remain to be addressed, however, before drawing any conclusion as to the therapeutic benefit of the pleiotropic effects of statins. For example, several preclinical studies indicate that the statins can be differentiated in terms of their nonlipid-related properties. However, there are, as yet, no comparative clinical trials of statins in the primary or secondary prevention of CAD that would allow us to determine the contribution of direct effects on the arterial wall to the therapeutic effect. Pharmacokinetic drug-drug interactions can also influence drug efficacy, tolerability, and compliance, and such interactions are both common and of more clinical relevance than often appreciated. Therefore, the different pharmacokinetic profiles among the statins should be carefully considered in order to understand the different spectrum of drug interactions, which are important determinants of safety in patients with hypercholesterolemia, especially in those requiring long-term therapy with drugs that are well-known CYP3A substrates and/or inhibitors.

References

Ahmad, S. (1990). Lovastatin: warfarin interaction. *Arch Intern Med 150*, 2407.

Aker, S., Ivens, K., Guo, Z., Grabensee, B., & Heering, P. (1998). Cardiovascular complications after renal transplantation. *Transplant Proc 30*, 2039–2042.

Aminou, P. N., van Vliet, A. K., van Erck, M., van Thiel, G. C. F., van Leeuwen, R. E. W., & Cohen, L. H. (1997). Inhibition of proliferation of human smooth muscle cells by various HMG-CoA reductase inhibi-

tors; comparison with other human cell types. *Biochim Biophys Acta 1345*, 259–268.

Anon (1998). FDA announce new drug-interaction warnings for mibefradil. *Am J Health Syst Pharm 55*, 210.

Appel, S., & Dingemanse, J. (1996). Clinical pharmacokinetics of fluvastatin with reference to other HMG-CoA reductase inhibitors. *Drugs Today 32*, 39–55.

Arnadottir, M., Eriksson, L.-O., Thysell, H., & Karkas, J. D. (1993). Plasma concentration profiles of simvastatin 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase inhibitory activity in kidney transplant recipients with and without ciclosporin. *Nephron 65*, 410–413.

Arnadottir, M., Eriksson, L.-O., Germershausen, J. I., & Thysell, H. (1994). Low-dose simvastatin is a well tolerated and efficacious cholesterol-lowering agent in ciclosporin-treated kidney transplant recipients: double-blind, randomized, placebo-controlled study in 40 patients. *Nephron 68*, 57–62.

Aviram, M., Dankner, G., Cogan, U., Hochgraf, E., & Brook, J. G. (1992). Lovastatin inhibits low-density lipoprotein oxidation and alters its fluidity and uptake by macrophages: in vitro and in vivo studies. *Metabolism 41*, 229–235.

Ayanian, J. Z., Fuchs, C. S., & Stone, R. M. (1988). Lovastatin and rhabdomyolisis. *Ann Intern Med 109*, 682–683.

Baetta, R., Donetti, E., Comparato, C., Calore, M., Rossi, A., Teruzzi, C., Paoletti, R., Fumagalli, R., & Soma, M. R. (1997a). In vitro and in vivo apoptosis by atorvastatin in stimulated smooth muscle cells. *Pharmacol Res 36*, 115–121.

Baetta, R., Paoletti, R., Fumagalli, R., & Soma, M. R. (1997b). Mevalonate modulation of cell proliferation and apoptosis. *Oncol Rep 4*, 257–261.

Bandoh, T., Mitani, H., Niihashi, M., Bandoh, T., Mitani, H., Niihashi, M., Kusumi, Y., Ishikawa, J., Kimura, M., Totsuka, T., Sakurai, I., & Hayashi, S. (1996). Inhibitory effect of fluvastatin at doses insufficient to lower serum lipids on the catheter-induced thickening of intima in rabbit femoral artery. *Eur J Pharmacol 315*, 37–42.

Bays, H. E., & Dujovne, C. A. (1998). Drug interactions of lipid-altering drugs. *Drug Safety 19*, 355–371.

Bellosta, S., Bernini, F., Ferri, N., Quarato, P., Canavesi, M., Arnaboldi, L., Fumagalli, R., Paoletti, R., & Corsini, A. (1998a). Direct vascular effects of HMG-CoA reductase inhibitors. *Atherosclerosis 137*(suppl.), S101–S109.

Bellosta, S., Via, D., Canavesi, M., Pfister, P., Fumagalli, R., Paoletti, R., & Bernini, F. (1998b). HMG-CoA reductase inhibitors reduce MMP-9 secretion by macrophages. *Arterioscler Thromb Vasc Biol 18*, 1671–1678.

Bernini, F., Didoni, G., Bonfadini, G., Bellosta, S., & Fumagalli, R. (1993). Requirement for mevalonate in acetylated LDL induction of cholesterol esterification in macrophages. *Atherosclerosis 104*, 19–26.

Bernini, F., Scurati, N., Bonfadini, G., & Fumagalli, R. (1995). HMG-CoA reductase inhibitors reduce acetyl LDL endocytosis in mouse peritoneal macrophages. *Arterioscler Thromb Vasc Biol 15*, 1352–1358.

Bertz, R. J., & Granneman, G. R. (1997). Use of in vitro and in vivo data to estimate the likelihood of metabolic pharmacokinetic interactions. *Clin Pharmacokinet 32*, 210–258.

Bocan, T. M. A., Mazur, M. J., Mueller, S. B., Brown, E. Q., Sliskovic, D. R., O'Brien, P. M., Creswell, M. W., Lee, H., Uhlendorf, P. D., & Roth, B. D. (1994). Antiatherosclerotic activity of inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A reductase in cholesterol-fed rabbits: a biochemical and morphological evaluation. *Atherosclerosis 111*, 127–142.

Bochaton Piallant, M. L., Gabbiani, F., Redard, M., Desmoulière, A., & Gabbiani, G. (1995). Apoptosis participates in cellularity regulation during rat aortic intimal thickening. *Am J Pathol 146*, 1059–1064.

Bourcier, T., Aikawa, M., & Libby, P. (1997). The HMG-CoA reductase inhibitors atorvastatin and lovastatin reduce accumulation of collagenase and 92-kD gelatinase released by cultured human monocytic and vascular smooth muscle cells. *Atherosclerosis 134*, 121.

Braun, W. E., & Marwick, T. H. (1994). Coronary artery disease in renal transplant recipients. *Cleve Clin J Med 61*, 370–385.

Brouwer, K. L. R., Dukes, G. E., & Powell, J. R. (1994). Influence of liver function on drug disposition. In W. E. Evans, J. J. Schentag, & W. J. Jusko (Eds.), *Applied Pharmacokinetics: Principles of Therapeutic Drug Monitoring*, 3rd Ed., Ch. 6 (pp. 6-1–6-59). Vancouver: Applied Therapeutics.

Brown, B. G., Zhao, X.-Q., Sacco, D. E., & Albers, J. J. (1993). Lipid lowering and plaque regression: new insights into prevention of plaque disruption and clinical events in coronary disease. *Circulation 87*, 1781–1791.

Brown, M. S., & Goldstein, J. L. (1991). Drugs used in the treatment of hyperlipoproteinemias. In A. G. Gilman, T. W. Rall, A. L. Nies, & P. Taylor (Eds.), *The Pharmacological Basis of Therapeutics* (pp. 874–896). Oxford: Pergamon Press.

Buchwald, H., Varco, R. L., Matts, J. P., Long, J. M., Fitch, L. L., Campbell, G. S., Pearce, M. B., Yellin, A. E., Edmiston, W. A., & Smik, R. D., Jr. (1990). Effect of partial ileal bypass surgery on mortality and morbidity from coronary heart disease in patients with hypercholesterolemia. Report of the Program on the Surgical Control of the Hyperlipidemias (POSCH). *N Engl J Med 323*, 946–955.

Cheng, H., Rogers, J. D., Sweany, A. E., Dobrinska, M. R., Stein, E. A., Tate, A. C., Amin, R. D., & Quan, H. (1992). Influence of age and gender on the plasma profiles of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitory activity following multiple doses of lovastatin and simvastatin. *Pharm Res 9*, 1629–1634.

Christians, U., Jacobsen, W., & Floren, L. C. (1998). Metabolism and drug interactions of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors in transplant patients: are the statins mechanistically similar? *Pharmacol Ther 80*, 1–34.

Cignarella, A., Brennhausen, B., von Eckardstein, A., Assmann, G., & Cullen, P. (1998). Differential effects of lovastatin on the trafficking of endogenous and lipoprotein-derived cholesterol in human monocyte-derived macrophages. *Arterioscler Thromb Vasc Biol 18*, 1322–1329.

Cilla, D. D., Whitfield, L. R., Gibson, D. M., Sedman, A. J., & Posvar, E. L. (1996). Multiple-dose pharmacokinetics, pharmacodynamics, and safety of atorvastatin, an inhibitor of HMG-CoA reductase, in healthy subjects. *Clin Pharmacol Ther 60*, 687–695.

Colli, S., Eligini, S., Lalli, M., Camera, M., Paoletti, R., & Tremoli, E. (1997). Vastatins inhibit tissue factor in cultured human macrophages. A novel mechanism of protection against atherothrombosis. *Arterioscler Thromb Vasc Biol 17*, 265–272.

Combalbert, J., Fabre, I., Fabre, G., Daleti, I., Delacourt, J., Cano, J. P., & Maurel, P. (1989). Metabolism and cyclosporine A. IV. Purification and identification of the rifampicin inducible human liver cytochrome P-450 (cyclosporine A oxidase) as a product of P450IIIA gene subfamily. *Drug Metab Dispos 17*, 197–207.

Corpier, C. L., Jones, P. H., Suki, W. N., Lederer, E. D., Quinones, M. A., Schmidt, S. W., & Young, J. B. (1988). Rhabdomyolysis and renal injury with lovastatin use. Report of two cases in cardiac transplant recipients. *JAMA 260*, 239–241.

Corsini, A., Mazzotti, M., Raiteri, M., Soma, M. R., Gabbiani, G., Fumagalli, R., & Paoletti, R. (1993). Relationship between mevalonate pathway and arterial myocyte proliferation: in vitro studies with inhibitors of HMG-CoA reductase. *Atherosclerosis 101*, 117–125.

Corsini, A., Maggi, F. M., & Catapano, A. L. (1995). Pharmacology of competitive inhibitors of HMG-CoA reductase. *Pharmacol Res 31*, 9–27.

Corsini, A., Bernini, F., Quarato, P., Donetti, E., Bellosta, S., Fumagalli, R., Paoletti, R., & Soma, M. R. (1996a). Non-lipid-related effects of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors. *Cardiology 87*, 458–468.

Corsini, A., Pazzucconi, F., Pfister, P., Paoletti, R., & Sirtori, C. R. (1996b). Inhibitor of proliferation of arterial smooth-muscle cells by fluvastatin. *Lancet 348*, 1584.

Corsini, A., Pazzucconi, F., Arnaboldi, L., Pfister, P., Fumagalli, R., Paoletti, R., & Sirtori, C. (1998). Direct effects of statins on the vascular wall. *J Cardiovasc Pharmacol 31*, 773–778.

Dain, J. G., Fu, E., Gorski, J., Nicoletti, J., & Scallen, T. J. (1993). Biotransformation of fluvastatin sodium in humans. *Drug Metab Dispos 21*, 567–572.

De Angelis, D. A., & Braun, P. E. (1996). Binding of 2',3'-cyclic nucleotide 3'-phosphodiesterase to myelin: an in vitro study. *J Neurochem 66*, 2523–2531.

Desager, J. P., & Horsmans, Y. (1996). Clinical pharmacokinetics of 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors. *Clin Pharmacokinet 31*, 348–371.

De Smedt, F., Boom, A., Pesesse, X., Schiffmann, N., & Erneux, C. (1996). Post-translational modification of human brain type I inositol-1,4,5-trisphosphate 5-phosphatase by farnesylation. *J Biol Chem 271*, 10419–10424.

Dollery, C. M., McEvan, J. R., & Henney, A. M. (1995). Matrix metalloproteinases and cardiovascular disease. *Circ Res 77*, 863–868.

Downs, J. R., Clearfield, M., Weis, S., Whitney, E., Shapiro, D. R., Beere, P. A., Langendorfer, A., Stein, E. A., Kruyer, W., & Gotto, A. M., Jr. (1998). Primary prevention of acute coronary events with lovastatin in men and women with average cholesterol levels: results of AFCAPS/TexCAPS. *JAMA 279*, 1615–1622.

Duell, P. B., Connor, W. E., & Illingworth, D. R. (1998). Rhabdomyolysis after taking atorvastatin with gemfibrozil. *Am J Cardiol 81*, 368–369.

Duggan, D. E., & Vickers, S. (1990). Physiological disposition of HMG-CoA reductase inhibitors. *Drug Metab Rev 22*, 333–362.

Dujovne, C. A., Chremos, A. N., Pool, J. L., Schnaper, H., Bradford, R. H., Shear, C. L., Higgins, J., Downton, M., Franklin, F. A., & Nash, D. T. (1991). Expanded clinical evaluation of lovastatin (EXCEL) study results. IV. Additional perspectives on the tolerability of lovastatin. *Am J Med 91*(suppl. 1B), 25S–30S.

East, C., Alivizatos, P. A., Grundy, S. M., Jones, P. H., & Farmer, J. A. (1988). Rhabdomyolysis in patients receiving lovastatin after cardiac transplantation. *N Engl J Med 318*, 47–48.

Endo, A. (1992). The discovery and development of HMG-CoA reductase inhibitors. *J Lipid Res 33*, 1569–1582.

Endres, M., Laufs, U., Huang, Z., Nakamura, T., Huang, P., Moskowitz, M. A., & Liao, J. K. (1998). Stroke protection by 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase inhibitors mediated by endothelial nitric oxide synthase. *Proc Natl Acad Sci USA 95*, 8880–8885.

Ernst, M. E., & Kelly, M. W. (1998). Mibefradil, a pharmacologically distinct calcium antagonist. *Pharmacotherapy 18*, 463–485.

Essig, M., Nguyen, G., Pri, D., Escoubet, B., Sraer, J. D., & Friedlander, G. (1998). 3-Hydroxy-3-methylglutaryl coenzyme A reductase inhibitors increase fibrinolytic activity in rat aortic endothelial cells. *Circ Res 83*, 683–690.

Falk, E., Shah, P. K., & Fuster, V. (1995). Coronary plaque disruption. *Circulation 92*, 657–671.

Feussner, G. (1994). HMG-CoA reductase inhibitors. *Curr Opin Lipidol 5*, 59–68.

Fischer, V., Johanson, L., Heitz, F., Tullman, R., Graham, E., Baldeck, J.-P., & Robinson, W. T. (1999). The HMG-CoA reductase inhibitor fluvastatin: effect on human cytochrome P450 and implications for metabolic drug interactions. *Drug Metab Dispos 27*, 410–416.

Garnett, W. R. (1995). Interactions with hydroxymethylglutaryl-coenzyme A reductase inhibitors. *Am J Health Syst Pharm 52*, 1639–1645.

Garnett, W. R., Venitz, J., Wilkens, R. C., & Dimenna, G. (1994). Pharmacokinetic effects of fluvastatin in patients chronically receiving digoxin. *Am J Med 96A*, 84S–86S.

Gaw, A., & Wosornu, D. (1992). Simvastatin during warfarin therapy in hyperlipoproteinaemia. *Lancet 340*, 979–980.

Gellman, J., Ezekowitz, M. D., Sarembock, I. J., Azrin, M. A., Nochomowitz, L. E., Lerner, E., & Haudenschild, C. C. (1991). Effect of lovastatin on intimal hyperplasia after balloon angioplasty. A study in an atherosclerotic hypercholesterolemic rabbit. *J Am Coll Cardiol 17*, 251–259.

Gibaldi, M. (1991). Pharmacokinetic variability-drug interactions. In M. Gibaldi (Ed.), *Biopharmaceutics and Clinical Pharmacokinetics*, 4th Ed. (pp. 305–343). Philadelphia: Lea & Febiger.

Giroux, L. M., Davignon, J., & Naruzewicz, M. (1993). Simvastatin inhibits the oxidation of low-density lipoproteins by activated human monocyte-derived macrophages. *Biochim Biophys Acta 1165*, 335–338.

Glomset, J. A., Gelb, M. H., & Farnsworth, C. C. (1990). Prenyl proteins in eukaryotic cells: a new type of membrane anchor. *Trends Biochem Sci 15*, 139–142.

Goldberg, R., & Roth, D. (1996). Evaluation of fluvastatin in the treatment of hypercholesterolemia in renal transplant recipients taking cyclosporine A. *Transplantation 62*, 1559–1564.

Goldstein, J. L., & Brown, M. S. (1990). Regulation of the mevalonate pathway. *Nature 343*, 425–430.

Gonzalez, J. M., & Badimon, L. (1996). Human and porcine smooth muscle cells share similar proliferation dependence on the mevalonate pathway: Implication for in vivo interventions in the porcine model. *Eur J Clin Invest 26*, 1023–1032.

Gonzalez, J. M., Vinals, M., Vidal, F., Llorente-Corts, V., & Badimon, L. (1997). Mevalonate deprivation impairs IGF-I/insulin signaling in human vascular smooth muscle cells. *Atherosclerosis 135*, 213–223.

Grunler, J., Ericsson, J., & Dallner, G. (1994). Branch-point reactions in the biosynthesis of cholesterol, dolichol, ubiquinone and prenylated proteins. *Biochim Biophys Acta 1212*, 259–277.

Guijarro, C., Blanco-Colio, L. M., Ortego, M., Alonso, C., Ortiz, A., Plaza, J. J., Diaz, C., Ernandez, G., & Egido, J. (1998). 3-Hydroxy-3-methylglutaryl coenzyme A reductase and isoprenylation inhibitors induce apoptosis of vascular smooth muscle cells in culture. *Circ Res 83*, 490–500.

Halstenson, C. E., Triscari, J., De Vault, A., Shapiro, B., Keane, W., & Pan, H. (1992). Single-dose pharmacokinetics of pravastatin and metabolites in patients with renal impairment. *J Clin Pharmacol 32*, 124–132.

Hamelin, B. A., & Turgeon, J. (1998). Hydrophilicity/lipophilicity: relevance for the pharmacology and clinical effects of HMG-CoA reductase inhibitors. *Trends Pharmacol Sci 19*, 26–37.

Han, D. K. M., Haudenschild, C. C., Hong, M. K., Tinkle, B. T., Leon, M. B., & Liau, G. (1995). Evidence for apoptosis in human atherogenesis and in rat vascular injury model. *Am J Pathol 147*, 267–277.

Haria, M., & McTavish, D. (1997). Pravastatin. A reappraisal of its pharmacological properties and clinical effectiveness in the management of coronary heart disease. *Drugs 53*, 299–336.

Havel, R. J., & Rapaport, E. (1995). Management of primary hyperlipidemia. *N Engl J Med 332*, 1301–1307.

Henwood, J. M., & Heel, R. C. (1995). Lovastatin. A preliminary review of its pharmacodynamic properties and therapeutic use in hyperlipidaemia. *Drugs 31*, 9–27.

Herd, J. A., Ballantyne, C. M., Farmer, J. A., Ferguson, J. J., III, Jones, P. H., West, M. S., & Gould, K. L. (1997). Effects of fluvastatin on coronary atherosclerosis in patients with mild to moderate cholesterol elevations (Lipoprotein and Coronary Atherosclerosis Study [LCAS]). *Am J Cardiol 80*, 278–286.

Hernandez-Perera, O., Perez-Sala, D., Navarro-Antolin, J., Sanchez-Pascuala, R., Hernandez, G., Diaz, C., & Lamas, S. (1998). Effects of the 3-hydroxy-3-methylglutaryl-CoA reductase inhibitors, atorvastatin and simvastatin, on the expression of endothelin-1 and endothelial nitric oxide synthase in vascular endothelial cells. *J Clin Invest 101*, 2711–2719.

Hirsh, J. (1991). Oral anticoagulant drugs. *N Engl J Med 324*, 1865–1875.

Hoffman, H. S. (1992). The interaction of lovastatin and warfarin. *Conn Med 56*, 107.

Holdaas, H., Hartmann, A., Stenstrom, J., Dahl, K., Borge, M., & Pfister, P. (1995). Effect of fluvastatin for safely lowering atherogenic lipids in renal transplant patients receiving cyclosporine A. *Am J Cardiol 76*, 102A–106A.

Holdaas, H., Fellström, B., & Holme, I. for the ALERT Study Group (1998). The ALERT trial: rationale and baseline data. In Abstracts of the XVII World Congress of the Transplantation Society, Montreal, July 12–17.

Horl, W. H., Riegel, W., Wanner, C., Haag-Weber, M., Schollmeyer, P., Wieland, H., & Wilms, H. (1989). Endocrine and metabolic abnormalities following kidney transplantation. *Klin Wochenschr 67*, 907–918.

Hsu, I., Spiler, S. A., & Johnson, N. E. (1995). Comparative evaluation of the safety and efficacy of HMG-CoA reductase inhibitor monotherapy

in the treatment of primary hypercholesterolemia. *Ann Pharmacother 29*, 743–759.

Hunninghake, D. B. (1992). HMG-CoA reductase inhibitors. *Curr Opin Lipidol 3*, 22–28.

Hussein, O., Schlezinger, S., Rosenblat, M., Keidar, S., & Aviram, M. (1997). Reduced susceptibility of low density lipoprotein (LDL) to lipid peroxidation after fluvastatin therapy in associated with the hypocholesterolemic effect of the drug and its binding to the LDL. *Atherosclerosis 128*, 11–18.

Igarashi, M., Takeda, Y., Mori, S., Ishibashi, N., Komatsu, E., Takahashi, K., Fuse, T., Yamamura, M., Kubo, K., Sugiyama, Y., & Saito, Y. (1997). Suppression of neointimal thickening by a newly developed HMG-CoA reductase inhibitor, BAYW6228 and its inhibitory effect on vascular smooth muscle cell growth. *Br J Pharmacol 120*, 1172–1178.

Inglese, J., Koch, W. J., Touhara, K., & Lefkowitz, R. J. (1995). Gβγ interaction with PH domains and Ras-MPK signaling pathways. *Trends Biochem Sci 20*, 151–156.

Isner, J. M., Kearney, M., Boartman, S., & Passeri, J. (1995). Apoptosis in human atherosclerosis and restenosis. *Circulation 91*, 2703–2711.

Jacobson, R. H., Wang, P., & Glueck, C. J. (1997). Myositis and rhabdomyolisis associated with concurrent use of simvastatin and nefazodone. *JAMA 277*, 296.

Jokubaitis, L. A. (1994). Updated clinical safety experience with fluvastatin. *Am J Cardiol 73*, 18D–24D.

Jones, K. J., Couldwell, W. T., Hinton, D. R., Su, Y., He, S., Anker, L., & Law, R. E. (1994). Lovastatin induces growth inhibition and apoptosis in human malignant glioma cells. *Biochem Biophys Res Commun 205*, 1681–1687.

Jukema, J. W., Bruschke, A. V. G., van Boven, A. J., Reiber, J. H., Bal, E. T., Zwinderman, A. H., Jansen, H., Boerma, G. J., van Rappard, F. M., & Lie, K. I. (1995). Effects of lipid lowering by pravastatin on progression and regression of coronary artery disease in symptomatic men with normal to moderately elevated serum cholesterol levels: The Regression Growth Evaluation Statin Study (REGRESS). *Circulation 91*, 2528–2540.

Kaesemeyer, W. H. (1999). Pravastatin sodium activates endothelial nitric oxide synthase independently of its cholesterol-lowering actions. *J Am Coll Cardiol 33*, 234–241.

Kandus, A., Kovac, D., Cerne, D., Koselj, M., Kaplan-Pavlovcic, S., Buturovic, J., Ponikvar, R., Kveder, R., Lindic, J., & Bren, A. F. (1998). Therapy of hyperlipidemia with lovastatin in kidney transplant patients on cyclosporine A immunosuppression: three-year experience. *Transplant Proc 30*, 1307–1309.

Kantola, T., Kivistö, K. T., & Neuvonen, P. J. (1998a). Pharmacokinetics and drug disposition. Grapefruit juice greatly increases serum concentrations of lovastatin and lovastatin acid. *Clin Pharmacol Ther 63*, 397–402.

Kantola, T., Kivistö, K. T., & Neuvonen, P. J. (1998b). Erythromycin and verapamil considerably increase serum simvastatin and simvastatin acid concentrations. *Clin Pharmacol Ther 64*, 177–182.

Kasiske, B. L., & Umen, A. J. (1987). Persistent hyperlipidemia in renal transplant patients. *Medicine 66*, 309–316.

Kitazawa, E., Tamura, N., Iwabuchi, H., Uchiyama, M., Muramatsu, S., Takahagi, H., & Tanaka, M. (1993). Biotransformation of pravastatin sodium. *Biochem Biophys Res Commun 192*, 597–602.

Kivistö, K. T., Kantola, T., & Neuvonen, P. J. (1998). Different effects of itraconazole on the pharmacokinetics of fluvastatin and lovastatin. *Br J Clin Pharmacol 46*, 49–53.

Kjekshus, J., Pedersen, T. R., & Tobert, J. A. (1996). Lipid-lowering therapy for patients with or at risk of coronary artery disease. *Curr Opin Lipidol 11*, 418–427.

Kline, S. S., & Harrell, C. C. (1997). Potential warfarin-fluvastatin interaction. *Ann Pharmacother 31*, 790.

Klintmalm, G. B. G., Iwatsuki, S., & Starzl, T. E. (1981). Cyclosporine A hepatotoxicity in 66 renal allograft recipients. *Transplantation 32*, 488–489.

Kockx, M. M., Cambier, B. A., Bortier, H. E., De Meyer, G. R., Declercq, S. C., Van Cauwelaert, P. A., & Bultick, J. (1994). Foam cell replication and smooth muscle cell apoptosis in human saphenous vein grafts. *Histopathology 25*, 365–371.

Laufs, U., La Fata, V., & Liao, J. K. (1997). Inhibition of 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase blocks hypoxia-mediated down-regulation of endothelial nitric oxide synthase. *J Biol Chem 272*, 31725–31729.

Laufs, U., La Fata, V., Plutzky, J., & Liao, J. K. (1998). Upregulation of endothelial nitric oxide synthase by HMG-CoA reductase inhibitors. *Circulation 97*, 1129–1135.

La Ville, A., Moshy, R., Turner, P. R., Miller, N. E., & Lewis, B. (1984). Inhibition of cholesterol synthesis reduces low-density-lipoprotein apoprotein B production without decreasing very-low-density-lipoprotein apoprotein B synthesis in rabbits. *Biochem J 218*, 321–323.

Laxminarayan, K. M., Chan, B. K., Tetaz, T., Bird, P. I., & Mitchell, C. A. (1994). Characterization of a cDNA encoding the 43-kDa membrane-associated inositol-polyphosphate 5-phosphatase. *J Biol Chem 269*, 17305–17310.

Lees, R. S., & Lees, A. M. (1995). Rhabdomyolysis from the coadministration of lovastatin and the antifungal agent itraconazole. *N Engl J Med 333*, 664–665.

Lennernas, H., & Fager, G. (1997). Pharmacodynamics and pharmacokinetics of the HMG-CoA reductase inhibitors. *Clin Pharmacokinet 32*, 403–425.

Liao, J. K., Zulueta, J. J., Feng-Sheng, Y., Peng, H., Cote, C. G., & Hassoun, P. M. (1995). Regulation of bovine endothelial constitutive nitric oxide synthase by oxygen. *J Clin Invest 96*, 2661–2666.

London, S. F., Gross, K. F., & Ringel, S. P. (1991). Cholesterol lowering agent myopathy. *Neurology 41*, 1159–1160.

Long-Term Intervention with Pravastatin in Ischaemic Disease (LIPID) Study Group (1998). Prevention of cardiovascular events and death with pravastatin in patients with coronary heart disease and a broad range of initial cholesterol levels. *N Engl J Med 339*, 1349–1357.

Mai, I., Bauer, S., & Fritsche, J. (1997). Clinical pharmacokinetics of a new HMG-CoA reductase inhibitor in renal transplant patients treated with cyclosporin A (CSA). *Eur J Clin Pharmacol 52*(suppl.), A137.

Maltese, W. A. (1990). Post-translational modification of proteins by isoprenoids in mammalian cells. *FASEB J 4*, 3319–3328.

Masaaki, K., Kurose, I., Russell, J., & Granger, D. N. (1997). Effect of fluvastatin on leukocyte-endothelial cell adhesion in hypercholesterolemic rats. *Arterioscler Thromb Vasc Biol 17*, 1521–1526.

McClellan, K. J., Wiseman, L. R., & McTavish, D. (1998). Cerivastatin. *Drugs 55*, 415–420.

Miller, D. B., & Spence, J. D. (1998). Clinical pharmacokinetics of fibric acid derivatives (fibrates). *Clin Pharmacokinet 34*, 155–162.

Mück, W., Ritter, W., Dietrich, H., Frey, R., & Kuhlmann, J. (1997a). Influence of the antacid Maalox and the H2-antagonist cimetidine on the pharmacokinetics of cerivastatin. *Int J Clin Pharmacol Ther 35*, 261–264.

Mück, W., Ritter, W., Frey, R., Wetzelsberger, N., Lucker, P. W., & Kuhlmann, J. (1997b). Influence of cholestyramine on the pharmacokinetics of cerivastatin. *Int J Clin Pharmacol Ther 35*, 250–254.

Mück, W., Ritter, W., Ochmann, K., Unger, S., Ahr, G., Wingender, W., & Kuhlmann, J. (1997c). Absolute and relative bioavailability of the HMG-CoA reductase inhibitor cerivastatin. *Int J Clin Pharmacol Ther 35*, 255–260.

Mück, W. (1998). Rational assessment of the interaction profile of cerivastatin supports its low propensity for drug interactions. *Drugs 56*(suppl. 1), 15–23.

Mück, W., Ochmann, K., Mazzu, A., & Lettieri, J. (1999). Biopharmaceutical profile of cerivastatin: a novel HMG-CoA reductase inhibitor. *Int J Med Res 27*, 107–114.

Munro, E., Patel, M., Chan, P., Betteridge, L., Clunn, G., Gallagher, K., Hughes, A., Schachter, M., Wolfe, J., & Sever, P. (1994). Inhibition of human vascular smooth muscle cell proliferation by lovastatin: the role of isoprenoid intermediates of cholesterol synthesis. *Eur J Clin Invest 24*, 766–772.

Neuvonen, P. J., Kantola, T., & Kivistö, K. T. (1998). Simvastatin but not

pravastatin is very susceptible to interaction with the CYP3A4 inhibitor itraconazole. *Clin Pharmacol Ther 63*, 332–334.

Nordin, C., Dahl, M. L., Eriksson, M., & Sjoberg, S. (1997). Is the cholesterol-lowering effect of simvastatin influenced by CYP2D6 polymorphism? *Lancet 350*, 29–30.

Norman, D. J., Illingworth, D. R., Munson, J., & Hosenpud, J. (1988). Myolysis and acute renal failure in heart-transplant patient receiving lovastatin. *N Engl J Med 318*, 46–47.

O'Driscoll, G., Green, D., & Taylor, R. R. (1997). Simvastatin, an HMG-coenzyme A reductase inhibitor, improves endothelial function within 1 month. *Circulation 95*, 1126–1131.

Olbricht, C., Wanner, C., Eisenhauer, T., Kliem, V., Doll, R., Boddaert, M., O'Grady, P., Krekler, M., Mangold, B., & Christians, U. (1997). Accumulation of lovastatin, but not pravastatin, in the blood of cyclosporine A-treated kidney graft patients after multiple doses. *Clin Pharmacol Ther 62*, 311–321.

Ong-Ajyooth, L., Ong-Ajyooth, S., Sirisalee, K., & Nilwarangkur, S. (1996). Lipoproteins and lipid peroxidation abnormalities in patients with chronic renal disease. *J Med Assoc Thai 79*, 505–512.

Packard, C. J., & West of Scotland Coronary Prevention Study Group (1998). Influence of pravastatin and plasma lipids on clinical events in the West of Scotland Coronary Prevention Study (WOSCOPS). *Circulation 97*, 1440–1445.

Pan, H. Y., DeVault, A. R., Swites, B. J., Whigan, D., Ivashkiv, E., Willard, D. A., & Brescia, D. (1990). Pharmacokinetics and pharmacodynamics of pravastatin alone and with cholestyramine in hypercholesterolemia. *Clin Pharmacol Ther 48*, 201–207.

Pan, H. Y., Triscar, J., Devault, A. R., Smith, S. A., Wangiverson, D., Swanson, B. N., & Willard, D. A. (1991). Pharmacokinetic interaction between propranolol and the HMG-CoA reductase inhibitors pravastatin and lovastatin. *Br J Clin Pharmacol 31*, 665–670.

Perez-Sala, D., & Mollinedo, F. (1994). Inhibition of isoprenoid biosynthesis induces apoptosis in human promyelocytic HL-60 cells. *Biochem Biophys Res Commun 199*, 1209–1215.

Perez-Sala, D., Collado-Escobar, D., & Mollinedo, F. (1995). Intracellular alkalination suppresses lovastatin-induced apoptosis in HL-60 cells through the inactivation of a pH-dependent indonuclease. *J Biol Chem 270*, 6235–6242.

Peters, T. K., Jewitt-Harris, J., Mehra, M., & Muratti, E. N. (1993). Safety and tolerability of fluvastatin with concomitant use of antihypertensive agents. An analysis of a clinical trial database. *Am J Hypertens 6*, 346S–352S.

Pierce, L. R., Wysowski, D. K., & Gross, T. P. (1990). Myopathy and rhabdomyolysis associated with lovastatin-gemfibrozil combination therapy. *JAMA 264*, 71–75.

Pogson, G. W., Kindred, L. H., & Carper, G. C. (1999). Rhabdomyolysis and renal failure associated with cerivastatin-gemfibrozil combination therapy. *Am J Cardiol 83*, 1146.

Posvar, E. L., Radulovic, L. L., Cilla, D. D., Whitfield, L. R., & Sedman, A. J. (1996). Tolerance and pharmacokinetics of single-dose atorvastatin, a potential inhibitor of HMG-CoA reductase, in healthy subjects. *J Clin Pharmacol 36*, 728–731.

Quion, J. A. V., & Jones, P. H. (1994). Clinical pharmacokinetics of pravastatin. *Clin Pharmacokinet 27*, 94–103.

Raiteri, M., Arnaboldi, L., McGeady, P., Gelb, M. H., Verri, D., Tagliabue, C., Quarato, P., Ferraboschi, P., Santaniello, E., Paoletti, R., Fumagalli, R., & Corsini, A. (1997). Pharmacological control of the mevalonate pathway: effect on arterial smooth muscle cell proliferation. *J Pharmacol Exp Ther 281*, 1144–1153.

Reaven, P., & Witztum, J. L. (1988). Lovastatin, nicotinic acid, and rhabdomyolysis. *Ann Intern Med 109*, 597–598.

Regazzi, M. B., Iacona, I., Campana, C., Raddato, V., Lesi, C., Perani, G., Gavazzi, A., & Vigano, M. (1993). Altered disposition of pravastatin following concomitant drug therapy with cyclosporine A in transplant recipients. *Transplant Proc 25*, 2732–2734.

Rettie, A. E., Korzekwa, K. R., Kunze, K. L., Lawrence, R. F., Eddy, A. C., Aoyama, T., Gelboin, H. V., Gonzalez, F. J., & Trager, W. F. (1992). Hydroxylation of warfarin by human cDNA-expressed cytochrome P-450: A role for P-4502C9 in the etiology of (*S*)-warfarin-drug interactions. *Chem Res Toxicol 5*, 54–59.

Richter, W. O., Jacob, B. G., & Schwandt, P. (1991). Interaction between fibre and lovastatin. *Lancet 338*, 706.

Riegger, G., Abletshauser, C., Ludwig, M., Schwandt, P., Widimsky, J., Weidinger, G., & Welzel, D. (1999). The effect of fluvastatin on cardiac events in patients with symptomatic coronary artery disease during one year of treatment. *Atherosclerosis 144*, 263–270.

Rosenson, R. S., & Tangney, C. (1998). Antiatherothrombotic properties of statins. *JAMA 279*, 1643–1650.

Sacks, F. M., Pfeffer, M. A., Moye, L. A., Rouleau, J. L., Rutherford, J. D., Cole, T. G., Brown, L., Warnica, J. W., Arnold, J. M., Wun, C. C., Davis, B. R., & Braunwald, E. (1996). The effect of pravastatin on coronary events after myocardial infarction in patients with average cholesterol levels. *N Engl J Med 335*, 1001–1009.

Sakai, M., Kobori, S., Matsumura, T., Biwa, T., Sato, Y., Takemura, T., Hakamata, H., Horiuchi, S., & Shichiri, M. (1997). HMG-CoA reductase inhibitors suppress macrophage growth induced by oxidized low density lipoprotein. *Atherosclerosis 133*, 51–59.

Scandinavian Simvastatin Survival Study Group (1994). Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: The Scandinavian Simvastatin Survival Study (4S). *Lancet 344*, 1383–1389.

Schmassmann-Suhijar, D., Bullingham, R., Gasser, R., Schmutz, J., & Haefeli, W. E. (1998). Rhabdomyolysis due to interaction of simvastatin with mibefradil. *Lancet 351*, 1929–1930.

Shepherd, J. (1995). Fibrates and statins in the treatment of hyperlipidaemia: an appraisal of their efficacy and safety. *Eur Heart J 16*, 5–13.

Shepherd, J., Cobbe, S. M., Ford, I., Isles, C. G., Lorimer, A. R., MacFarlane, P. W., McKillop, J. H., & Packard, C. J. (1995). Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia. *N Engl J Med 333*, 1301–1307.

Singhvi, S. M., Pan, H. Y., Morrison, R. A., & Willard, D. A. (1990). Disposition of pravastatin sodium, a tissue-selective HMG-CoA reductase inhibitor, in healthy subjects. *Br J Clin Pharmacol 29*, 239–243.

Smith, H. T., Jokubaitis, L. A., Troendle, A. J., Hwang, D. S., & Robinson, W. T. (1993). Pharmacokinetics of fluvastatin and specific drug interactions. *Am J Hypertens 6*, 375S–382S.

Smith, P. F., Eydelloth, R. S., Grossman, S. J., Stubbs, R. J., Schwartz, M. S., Germershausen, J. I., Vyas, K. P., Kari, P. H., & MacDonald, J. S. (1991). HMG-CoA reductase inhibitor-induced myopathy in the rat: cyclosporine A interaction and mechanism studies. *J Pharmacol Exp Ther 257*, 1225–1235.

Soma, M. R., Donetti, E., Parolini, C., Mazzini, G., Ferrari, C., Fumagalli, R., & Paoletti, R. (1993). HMG-CoA reductase inhibitors: in vivo effects on carotid intimal thickening in normocholesterolemic rabbits. *Arterioscler Thromb Vasc Biol 13*, 571–578.

Soma, M. R., Baetta, R., De Renzis, S., Mazzini, G., Davegna, C., Magrassi, L., Butti, G., Pezzotta, S., Paoletti, R., & Fumagalli, R. (1995a). In vitro and in vivo enhanced anti-tumor activity of carmustine (BCNU) by simvastatin, a hypocholesterolemic drug. *Cancer Res 55*, 597–602.

Soma, M. R., Parolini, C., Donetti, E., Fumagalli, R., & Paoletti, R. (1995b). Inhibition of isoprenoid biosynthesis and arterial smooth muscle cell proliferation. *J Cardiovasc Pharmacol 25*, S20–S24.

Spach, D. H., Bauwens, J. E., Clark, C. D., & Burke, W. G. (1991). Rhabdomyolysis associated with lovastatin and erythromycin use. *West J Med 154*, 213–215.

Spence, J. D., Munoz, C. E., Hendicks, L., Latchinian, L., & Khouri, H. E. (1995). Pharmacokinetics of the combination of fluvastatin and gemfibrozil. *Am J Cardiol 76*, 80A–83A.

Stern, R., Abel, R., Gibson, G. L., & Besserer, J. (1997). Atorvastatin does not alter the anticoagulant activity of warfarin. *J Clin Pharmacol 37*, 1062–1064.

Stern, R. H., Gibson, D. M., & Whitfield, L. R. (1998). Cimetidine does not alter atorvastatin pharmacokinetics or LDL-cholesterol reduction. *Eur J Clin Pharmacol 53*, 475–478.

Stockley, I. H. (1996). *Drug Interactions. A Source Book of Adverse Interactions, Their Mechanisms, Clinical Importance and Management.* London: Pharmaceutical Press.

Tal, A., Rajeshawari, M., & Isley, W. (1997). Rhabdomyolysis associated with simvastatin-genfibrozil therapy. *South Med J 90*, 546–547.

Tapon, N., & Hall, A. (1997). Rho Rac and Cdc42 GTPases regulate the organization of the actin cytoskeleton. *Curr Opin Cell Biol 9*, 86–92.

Thummel, K. E., & Wilkinson, G. R. (1998). In vitro and in vivo drug interaction involving human CYP3A. *Annu Rev Pharmacol Toxicol 38*, 389–430.

Tobert, J. A. (1988). Efficacy and long-term adverse effect pattern of lovastatin. *Am J Cardiol 62*, 28J–34J.

Transon, C., Leemann, T., Vogt, N., & Dayer, P. (1995). In vivo inhibition profile of cytochrome P450 tb (CYP2C9) by (±)-fluvastatin. *Clin Pharmacol Ther 58*, 412–417.

Trilli, L. E., Kelley, C. L., Aspinall, S. L., & Kroner, B. A. (1996). Potential interaction between warfarin and fluvastatin. *Ann Pharmacother 30*, 1399–1402.

Tse, F. L. S., Jaffe, J. M., & Troendle, A. (1992). Pharmacokinetics of fluvastatin after single and multiple doses of normal volunteers. *J Clin Pharmacol 32*, 630–638.

Tse, F. L. S., Nickerson, D. F., & Yardley, W. S. (1993). Binding of fluvastatin to blood cells and plasma proteins. *J Pharm Sci 82*, 942–947.

Umetani, N., Kanayama, Y., Okamura, M., Negoro, N., & Takeda, T. (1996). Lovastatin inhibits gene expression of type-I scavenger receptor in THP-1 human macrophages. *Biochim Biophys Acta 1303*, 199–206.

Vanhaecke, J., Van Cleemput, J., Van Lierde, J., Daenen, W., & De Geest, H. (1994). Safety and efficacy of low dose simvastatin in cardiac transplant recipients treated with cyclosporine A. *Transplantation 58*, 42–45.

Wacher, V. J., Wu, C. Y., & Benet, L. Z. (1995). Overlapping substrate specificities and tissue distribution of cytochrome P450 3A and P-glycoprotein: implications for drug delivery and activity in cancer chemotherapy. *Mol Carcinogen 13*, 129–134.

Walker, J. F. (1989). Simvastatin: the clinical profile. *Am J Med 87* (suppl. 4A), 44S–46S.

Wang, R. W., Karl, P. H., Lu, A. Y. H., Thomas, P. E., Guengerich, F. P., & Vyas, K. P. (1991). Biotransformation of lovastatin. IV. Identification of cytochrome P450 3A proteins as the major enzymes responsible for the oxidative metabolism of lovastatin in rat and human liver microsomes. *Arch Biochem Biophys 290*, 355–361.

Wilkinson, G. R. (1996). Cytochrome P4503A (CYP3A) metabolism: prediction of in vivo activity in humans. *J Pharmacokinet Biopharm 24*, 475–489.

Williams, J. K., Sukhova, G. K., Herrington, D. M., & Libby, P. (1998). Pravastatin has cholesterol-lowering independent effects on the artery wall of atherosclerotic monkeys. *J Am Coll Cardiol 31*, 684–691.

Wrighton, S. A., VandenBranden, M., & Ring, B. J. (1996). The human drug metabolizing cytochromes P450. *J Pharmacokinet Biopharm 24*, 461–473.

Yang, B. B., Hounslow, N. J., Sedman, A. J., & Forgue, S. T. (1996). Effects of atorvastatin, an HMG-CoA reductase inhibitor, on hepatic oxidative metabolism of antipyrine. *J Clin Pharmacol 36*, 356–360.

Yoshimura, N., Oka, T., Okamoto, M., & Ohmori, Y. (1992). The effects of pravastatin on hyperlipidemia in renal transplant recipients. *Transplantation 53*, 94–99.

Zhang, F. L., & Casey, P. J. (1996). Protein prenylation: molecular mechanisms and functional consequences. *Annu Rev Biochem 65*, 241–269.

Zhu, B. Q., Sievers, R. E., Sun, Y. P., Isenberg, W. M., & Parmley, W. W. (1992). Effect of lovastatin on suppression and regression of atherosclerosis in lipid-fed rabbits. *J Cardiovasc Pharmacol 19*, 246–255.

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PHARMACOKINETICS AND DISPOSITION

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Effect of itraconazole on cerivastatin pharmacokinetics

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Abstract *Objective*: To determine the effects of itraconazole, a potent inhibitor of CYP3A4, on the pharmacokinetics of cerivastatin, a competitive 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor.
Methods: A randomized, double-blind, cross-over study design with two phases, which were separated by a washout period of 4 weeks, was used. In each phase ten healthy volunteers took 200 mg itraconazole or matched placebo orally once daily for 4 days according to a randomization schedule. On day 4, 0.3 mg cerivastatin was administered orally. Serum concentrations of cerivastatin, its major metabolites, active and total HMG-CoA reductase inhibitors, itraconazole and hydroxyitraconazole were measured up to 24 h.
Results: Itraconazole increased the area under the concentration-time curve from time zero to infinity ($AUC_{0-\infty}$) of the parent cerivastatin by 15% ($P < 0.05$). The mean peak serum concentration (C_{max}) of cerivastatin lactone was increased 1.8-fold (range 1.1-fold to 2.4-fold, $P < 0.001$) and the $AUC_{0-24\ h}$ 2.6-fold (range 2.0-fold to 3.6-fold, $P < 0.001$) by itraconazole. The elimination half-life ($t_{1/2}$) of cerivastatin lactone was increased 3.2-fold ($P < 0.001$). Itraconazole decreased the $AUC_{0-24\ h}$ of the active M-1 metabolite of cerivastatin by 28% ($P < 0.05$), whereas the $AUC_{0-24\ h}$ of the more active metabolite, M-23, was increased by 36% ($P < 0.05$). The $AUC_{0-24\ h}$ and $t_{1/2}$ of active HMG-CoA reductase inhibitors were increased by 27% ($P < 0.05$) and 40% ($P < 0.05$), respectively, by itraconazole.
Conclusions: Itraconazole has a modest interaction with cerivastatin. Inhibition of the CYP3A4-mediated M-1 metabolic pathway leads to elevated serum concentrations of cerivastatin, cerivastatin lactone and metabolite M-23, resulting in increased concentrations of active HMG-CoA reductase inhibitors.

Key words Cerivastatin · Itraconazole · Interaction

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Introduction

Cerivastatin is a new inhibitor of HMG-CoA reductase, the rate-limiting step in cholesterol biosynthesis [1]. The bioavailability of cerivastatin is about 60% after oral administration [2]. Cerivastatin is administered as the active hydroxyacid form, cerivastatin acid, and it is eliminated via cytochrome P-450 (CYP) mediated biotransformation. Two major (M-1 and M-23) and one minor (M-24) active metabolites are formed in the liver. The formation of the major active metabolites is via two equally important metabolic pathways, which produce the demethylation product M-1 and the hydroxylation product M-23 [3]. M-23 and M-24 exhibit 100% and M-1 exhibits 30–50% of the HMG-CoA reductase inhibitory activity of cerivastatin [4]. In vitro studies with human liver microsomes suggest that the demethylation pathway (formation of M-1), but not the hydroxylation pathway (formation of M-23), is mediated by CYP3A enzymes [3]. Cerivastatin and its metabolites are in equilibrium with their inactive lactone forms [5].

The antimycotic itraconazole, a potent inhibitor of CYP3A4, increases serum concentrations of simvastatin and lovastatin about ten- to 30-fold [6, 7]. Prolonged high serum concentrations of lovastatin and simvastatin enhance the risk of skeletal muscle injury and can result in severe rhabdomyolysis even within 2 weeks [8–10]. We wanted to study the effect of itraconazole on the

pharmacokinetics of cerivastatin in healthy volunteers to characterize the susceptibility of cerivastatin to interact with CYP3A4 inhibitors.

Methods

Study design

Seven female and three male healthy volunteers (age range 18–28 years, weight range 52–90 kg) participated in the study after giving written informed consent (Table 1). All subjects were ascertained to be healthy by means of a physical examination and laboratory tests (e.g. blood haemoglobin, serum creatinine, alanine aminotransferase, creatine kinase). Five female volunteers were using oral contraceptive steroids but the other volunteers were not using any continuous medications. One female subject was a smoker. The study protocol was approved by the Ethics Committee of the Department of Clinical Pharmacology, University of Helsinki, and by the Finnish National Agency for Medicines.

A randomized, double-blind, two-phase cross-over study design with an interval of 4 weeks between the phases was used. In each phase the volunteers took 200 mg itraconazole (Sporanox, Janssen, Beerse, Belgium) or matched placebo orally once daily at 0800 hours for 4 days, according to a randomization schedule. On day 4, a single oral dose of 0.3 mg cerivastatin (1 Lipobay 0.3 mg tablet, Bayer, Leverkusen, Germany) was administered with 150 ml water at 0900 hours. The volunteers fasted for 1 h before administration of cerivastatin and had a hot standard meal 4 h and a light standard meal 8 h afterwards.

Blood sampling and determination of drug concentrations

On day 4, timed blood samples (15 ml each) were drawn into siliconized Venoject tubes (Terumo Europe, Leuven, Belgium) immediately before and 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12 h and 24 h after the administration of cerivastatin. Serum was separated within 30 min and stored at −70 °C until analysis.

Determination of cerivastatin and its metabolites by liquid chromatography-mass spectrometry (LC/MS/MS)

The concentrations of cerivastatin and its metabolites, cerivastatin lactone, M-1, M-23 and M-24 acids and lactones were quantified by LC/MS/MS. Using deuterated cerivastatin and deuterated cerivastatin lactone as internal standards for the respective acids and lactones, peak area ratios were calculated for quantification. The analytes containing the internal standards in serum were extracted into methyl tert-butyl ether. After evaporation to dryness, the sample was reconstituted with 50:50 acetonitrile:ammonium formate (10 mM, pH 4.0) and analysed by liquid chromatography/Turbo Ion Spray tandem mass spectrometry in the positive ion mode. The LC/MS/MS system was equipped with two LC10AD pumps (Shimadzu, Columbia, Mo., USA), a YMC Basic guard column (10 × 2 mm) and a YMC Basic analytical column (50 × 2 mm, 5 μm) and a PE-SCIEX API 365 mass spectrometer (PE-SCIEX, Concord, Ontario, Canada). A gradient elution using a mobile phase A, containing 100% acetonitrile, and a mobile phase B, containing 100% ammonium formate (pH 4.0) were used as follows: At 0.0 min 60% A, at 0.3 min 100% A, at 1.0 min 100% A, at 1.1 min 60% A and at 3.5 min 60% A. The following $[M + H]^+$ precursor and product ions were used in the selected reaction monitoring (SRM) mode: cerivastatin (460 → 356), cerivastatin lactone (442 → 354), M-1 acid (446 → 342), M-1 lactone (428 → 340), M-23 acid (476 → 340), M-23 lactone (458 → 338), M-24 acid (462 → 340), and M-24 lactone (444 → 338). The lower limit of quantification (LLQ) was 0.01 $ng \cdot ml^{-1}$ for cerivastatin and its lactone, 0.05 $ng \cdot ml^{-1}$ for M-1 acid and its lactone, 0.1 $ng \cdot ml^{-1}$ for M-23 acid and its lactone and 0.5 $ng \cdot ml^{-1}$ for M-24 acid and its lactone. The inter-assay and intra-assay coefficients of variation (CV) were less than 20% for all the analytes.

Determination of active and total HMG-CoA reductase inhibitors by radioenzyme inhibition assay

Serum concentrations of cerivastatin (acid) equivalents (cerivastatin and active metabolites) were determined both before and after base hydrolysis of the lactones using radioenzyme inhibition assay (REA) [11, 12]. The inhibition of the HMG-CoA reductase was measured in a reaction mixture containing rat liver microsomes, NADPH-regenerating cofactors, substrate (^{14}C-HMG-CoA) and the serum specimen. After terminating the reaction with HCl, an internal standard (^{3}H-mevalonolactone) was added. ^{14}C-mevalonolactone was separated from unreacted substrate using anion exchange chromatography (AG 1-X8 resin). The ^{14}C-mevalonolactone radioactivity was counted in the eluent. Concentrations are reported as ng-equivalents of cerivastatin per millilitre. The LLQ in REA before hydrolysis (active) was 0.8 ng-equivalents of cerivastatin per millilitre, and after hydrolysis (total) the LLQ was 1.0 ng-equivalents of cerivastatin per millilitre. The inter-assay CV was less than 10% at relevant concentrations.

Table 1 Characteristics of the subjects

Subject number	Sex	Age (years)	Weight (kg)	Smoker	User of oral contraceptives?	Itraconazole $AUC_{0-24\ h}$[a]	Hydroxy-itraconazole $AUC_{0-24\ h}$[a]
1	Female	22	59	No	Cyproterone acetate 2 mg + Ethinylestradiol 35 μg	4.5	10.8
2	Male	22	74	No	No	3.9	9.1
3	Male	24	78	No	No	3.6	8.7
4	Female	18	52	No	No	3.9	13.6
5	Female	24	55	Yes	Norgestimate 250 μg + Ethinylestradiol 35 μg	6.7	13.5
6	Female	20	61	No	Desogestrel 0.15 mg + Ethinylestradiol 20 μg	6.2	15.0
7	Female	21	54	No	Desogestrel 0.15 mg + Ethinylestradiol 20 μg	4.6	11.1
8	Female	21	55	No	Gestodene 75 μg + Ethinylestradiol 20 μg	6.2	14.6
9	Male	28	90	No	No	5.8	11.1
10	Female	19	57	No	No	9.6	23.4

[a] $AUC_{0-24\ h}$, area under the serum concentration-time curve from 0 to 24 h ($\mu g \cdot ml^{-1} \cdot h$) after the administration of cerivastatin

Determination of itraconazole and hydroxyitraconazole by high-performance liquid chromatography

The concentrations of itraconazole and hydroxyitraconazole were determined by high-performance liquid chromatography (HPLC) using fluorescence detection [7, 13]. The detection limit was 10 ng · ml^{-1} for both compounds. The interday CV for itraconazole was 4.8% and for hydroxyitraconazole 5.6% at relevant concentrations.

Pharmacokinetic calculations

The pharmacokinetics of the drugs were characterized, as appropriate, by peak concentration in serum (C_{max}), concentration peak time (t_{max}), elimination half-life ($t_{1/2}$), and areas under the concentration-time curve from time zero up to 24 h ($AUC_{0-24\ h}$) and to infinity ($AUC_{0-\infty}$). The elimination rate constant (k_{el}) was determined by a linear regression analysis of the log-linear phase of the serum drug concentration-time curve. The $AUC_{0-\infty}$ was calculated by use of the trapezoidal rule with extrapolation to infinity by dividing the last measured concentration by k_{el}. The $t_{1/2}$ was calculated from $t_{1/2} = \ln 2/k_{el}$. The $AUC_{0-24\ h}$ of itraconazole and hydroxyitraconazole refers to the time between 0 and 24 h after the ingestion of cerivastatin, i.e. between 1 and 25 h after the last dose of the pretreatment.

Statistical analysis

The data are expressed as mean values with (SD) in the text and as mean values with (SEM) in the figures. Data were analysed by the Student's *t*-test for paired values or, in the case of t_{max}, by the Wilcoxon signed rank test. The pharmacokinetic variables were log-transformed before statistical analysis when appropriate. The power of the study to detect a 30% difference in cerivastatin C_{max} and $AUC_{0-\infty}$ between the itraconazole and placebo phases was 80% at the 5% level of significance. Differences were regarded statistically significant when P was < 0.05.

Results

Cerivastatin and cerivastatin lactone

The effect of itraconazole on the $AUC_{0-24\ h}$, C_{max}, t_{max} and $t_{1/2}$ of cerivastatin was not statistically significant (Fig. 1, Table 2). However, the $AUC_{0-\infty}$ of cerivastatin was increased by 15% [from 23.8 (12.0) ng · ml^{-1} · h to 27.5 (14.6) ng · ml^{-1} · h; $P < 0.05$) compared with placebo.

The mean C_{max} and $AUC_{0-24\ h}$ of cerivastatin lactone were increased 1.8-fold and 2.6-fold, respectively ($P < 0.001$; Fig. 1, Table 2) and the mean $t_{1/2}$ 3.2-fold ($P < 0.001$) by itraconazole. All these variables of cerivastatin lactone were increased in each of the ten subjects, the increase ranging from 1.1-fold to 2.4-fold for C_{max}, from 2.0-fold to 3.6-fold for $AUC_{0-24\ h}$ and from 1.8-fold to 5.2-fold for $t_{1/2}$. The t_{max} of cerivastatin lactone was significantly later for the itraconazole phase than for the placebo phase (4 h vs 2.5 h, $P < 0.05$).

Metabolites of cerivastatin

Itraconazole decreased the mean $AUC_{0-24\ h}$ of metabolite M-1 by 28% ($P < 0.05$) compared with placebo, while the mean $AUC_{0-24\ h}$ of metabolite M-23 was increased by 36% ($P < 0.05$) (Fig. 1, Table 2). The C_{max}, t_{max} and $t_{1/2}$ of M-1 and M-23 were not significantly affected by itraconazole. The concentrations of metabolite M-24 and its lactone were below the LLQ (0.5 ng · ml^{-1}) during both the itraconazole and the placebo phases.

Active and total HMG-CoA reductase inhibitors

The mean $AUC_{0-24\ h}$ of active HMG-CoA reductase inhibitors was increased by 27% ($P < 0.05$) and the $t_{1/2}$ by 40% ($P < 0.05$) by itraconazole (Fig. 2, Table 2). The C_{max} and t_{max} of active HMG-CoA reductase inhibitors were not significantly altered by itraconazole. The pharmacokinetic variables of total HMG-CoA reductase inhibitors were not significantly affected by itraconazole, except for t_{max}, which was increased from the control value of 3 h (median) to 4 h ($P < 0.05$) by itraconazole.

Table 2 The pharmacokinetic variables of cerivastatin, cerivastatin lactone, metabolite M-1, metabolite M-23 and active and total HMG-CoA reductase inhibitors in ten subjects following pretreatment with 200 mg itraconazole or placebo once daily for 4 days. Data are mean values with (SD); t_{max} data are given as median with (range)

	C_{max} (ng · ml^{-1})		t_{max} (h)		$t_{1/2}$ (h)		$AUC_{0-24\ h}$ (ng · ml^{-1} · h)	
	Itraconazole	Placebo	Itraconazole	Placebo	Itraconazole	Placebo	Itraconazole	Placebo
Cerivastatin	3.9 (1.6)	3.8 (1.2)	2.5 (1–4)	2.3 (1.5–3)	4.3 (1.1)	4.0 (1.4)	26.7 (13.7)	23.3 (11.0)
Cerivastatin lactone	0.3 (0.1)*	0.2 (0.1)	4 (3–6)**	2.5 (2–4)	9.8 (2.7)*	3.1 (1.3)	2.9 (0.8)*	1.1 (0.4)
M-1	0.2 (0.1)	0.3 (0.1)	4 (2.5–6)	3.5 (2.5–6)	6.8 (3.5)‡	5.0 (3.6)#	1.2 (0.9)**	1.6 (1.3)
M-23	0.8 (0.3)	0.7 (0.3)	6 (3–12)	5 (2.5–10)	8.8 (5.1)#	7.9 (5.5)‡	8.1 (3.4)**	5.9 (2.3)
Active HMG-CoA reductase inhibitors	4.6 (1.8)	4.5 (1.7)	4 (2.5–6)	3.5 (2.5–6)	4.0 (1.5)**	2.8 (0.7)	38.7 (21.0)**	30.6 (14.4)
Total HMG-CoA reductase inhibitors	4.7 (1.7)	4.6 (1.5)	4 (2.5–6)**	3 (2.5–6)	4.1 (1.9)	3.8 (2.4)#	38.9 (23.1)	34.0 (17.8)

* $P < 0.001$ versus placebo phase
** $P < 0.05$ versus placebo phase
‡ Based on values of eight subjects
# Based on values of nine subjects

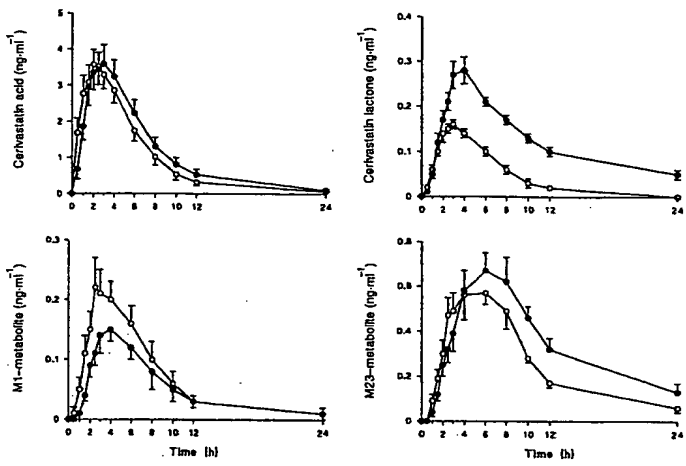

Fig. 1 Mean with (SEM) serum concentrations of cerivastatin acid and lactone (*upper panel*) and metabolite M1 and M23 (*lower panel*) in ten healthy volunteers after a single oral dose of 0.3 mg cerivastatin, following daily use of oral itraconazole (200 mg, *solid circles*) or placebo (*open circles*) for 4 days. The concentrations below the lower limits of quantification were taken as zero in the calculation of the mean values

Itraconazole and hydroxyitraconazole

There was a fourfold and threefold interindividual variation in the C_{max} and $AUC_{0-24\,h}$ of itraconazole, respectively (Table 1). The mean $AUC_{0-24\,h}$ of itraconazole was 5.5 (1.8) $\mu g \cdot ml^{-1} \cdot h$ and that of hydroxyitraconazole 13.1 (4.2) $\mu g \cdot ml^{-1} \cdot h$.

Discussion

To avoid predisposition of healthy volunteers to multiple doses of potentially toxic drug combinations, we used a single-dose administration of cerivastatin preceded by a relatively short pretreatment with itraconazole. The results of this study demonstrate that a 4-day pretreatment with a daily dose of 200 mg of itraconazole has an effect on the pharmacokinetics of cerivastatin. The serum concentrations of cerivastatin and its lactone, metabolite M-23 and active HMG-CoA reductase inhibitors were increased by itraconazole. Correspondingly, the serum concentration of metabolite M-1 was reduced. Although the effect was modest, the interaction could be greater after a longer use or a higher dosage of itraconazole.

In the present study, the concentrations of parent cerivastatin, its active metabolites M-1 and M-23 and cerivastatin lactone were measured by using a sensitive and specific LC/MS/MS method. Furthermore, the concentrations of active and total HMG-CoA reductase inhibitors were determined by using radioenzyme inhibition assay [11, 12]. The magnitude of the increase observed in the $AUC_{0-24\,h}$ of active HMG-CoA reductase inhibitors during the itraconazole phase was in good agreement with the serum concentrations of cerivastatin and its active metabolites.

These data indirectly suggest that CYP3A4 plays a role in the formation of M-1 from cerivastatin, whereas other enzymes are likely to be mainly responsible for the M-23 metabolic pathway. This notion is in good agreement with recent in vitro studies with human liver microsomes by Boberg et al., which suggest that only the

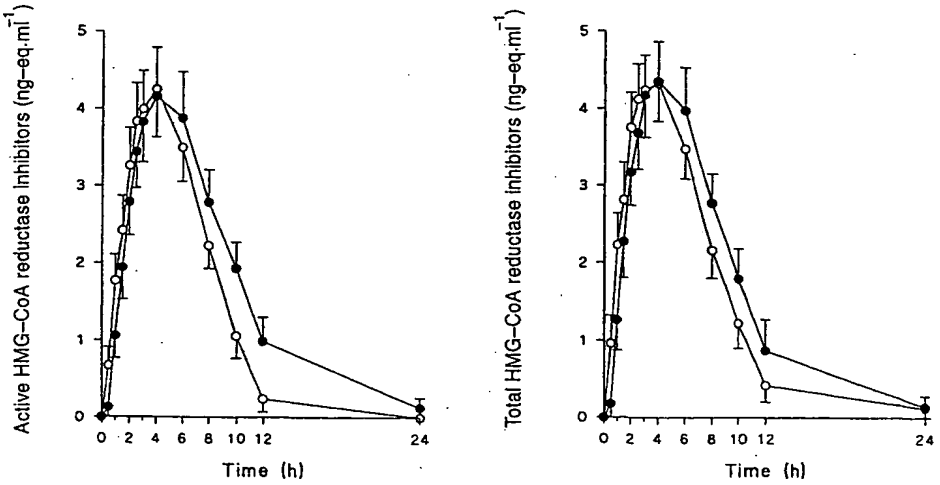

Fig. 2 Mean with (SEM) serum concentrations of active and total HMG-CoA reductase inhibitors in ten healthy volunteers after a single oral dose of 0.3 mg cerivastatin following daily use of oral itraconazole (200 mg, *solid circles*) or placebo (*open circles*) for 4 days. Ng-eq refers to the ng-equivalents of cerivastatin. The concentrations below the lower limits of quantification were taken as zero in the calculation of the mean values

demethylation pathway (formation of M-1), and not the hydroxylation pathway (formation of M-23) is mediated by CYP3A4 [3].

Concomitant administration of itraconazole with lovastatin or simvastatin, which have an extensive first-pass metabolism and are substrates of CYP3A4, leads to a greater than tenfold increase in the concentrations of these statins in the peripheral blood and a substantially enhanced risk of skeletal muscle toxicity [6–10, 14, 15]. Atorvastatin is also metabolized by CYP3A4 and its serum concentrations are increased by concomitant use of itraconazole [16, 17]. However, pravastatin and fluvastatin are eliminated mainly by mechanisms other than CYP3A4-mediated biotransformation and their serum concentrations are not increased when co-administered with itraconazole [6, 18–21]. In the present study, an interaction was found between itraconazole and cerivastatin, the magnitude of which is clearly less than the interactions observed when itraconazole was co-administered with lovastatin, simvastatin or even atorvastatin [6, 7, 17]. The present findings are well in line with the results of a recent study by Mück et al., in which erythromycin 500 mg t.i.d. only slightly affected the pharmacokinetics of cerivastatin and its metabolites [22].

In conclusion, itraconazole has a modest interaction with cerivastatin. Inhibition of the CYP3A4-mediated M-1 metabolic pathway leads to elevated serum concentrations of cerivastatin, cerivastatin lactone and metabolite M-23, resulting in increased concentrations of active HMG-CoA reductase inhibitors.

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References

1. Stein E, Sprecher D, Allenby KS, Tosiello RL (1997) Cerivastatin, a new potent synthetic HMG-CoA reductase inhibitor: Effect of 0.2 mg daily in subjects with primary hypercholesterolemia. J Cardiovasc Pharmacol 2: 7–16
2. Mück W, Ritter W, Ochmann K, Unger S, Ahr G, Wingender W, Kuhlmann J (1997) Absolute and relative bioavailability of the HMG-CoA reductase inhibitor cerivastatin. Int J Clin Pharmacol Ther 35: 255–260
3. Boberg M, Angerbauer R, Fey P, Kanhai WK, Karl W, Kern A, Ploschke J, Radtke M (1997) Metabolism of cerivastatin by human liver microsomes in vitro. Characterization of primary metabolic pathways and of cytochrome P450 isozymes involved. Drug Metab Dispos 25: 321–331
4. Bischoff H, Angerbauer R, Boberg M, Schmidt D (1997) Cerivastatin: High enzyme affinity and active metabolites contribute to its high pharmacological activity. Atherosclerosis 130 [Suppl]: S25
5. Cheng H, Jusko WJ (1993) Pharmacokinetics of reversible metabolic systems. Biopharm Drug Dispos 14: 721–766
6. Neuvonen PJ, Kantola T, Kivistö KT (1998) Simvastatin but not pravastatin is very susceptible to interaction with the CYP3A4 inhibitor itraconazole. Clin Pharmacol Ther 63: 332–341
7. Neuvonen PJ, Jalava K-M (1996) Itraconazole drastically increases plasma concentrations of lovastatin and lovastatin acid. Clin Pharmacol Ther 60: 54–61
8. Lees RS, Lees AM (1995) Rhabdomyolysis from the coadministration of lovastatin and the antifungal agent itraconazole. N Engl J Med 333: 664–665
9. Horn M (1996) Coadministration of itraconazole with hypolipidemic agents may induce rhabdomyolysis in healthy individuals. Arch Dermatol 132: 1254
10. Segaert MF, De Soete C, Vandewiele I, Verbanck J (1996) Drug-interaction-induced rhabdomyolysis. Nephrol Dial Transplant 11: 1846–1847
11. Manning JA, Swanson BN, Emaus NA, Fry J (1989) A semi-automated, radioenzymatic assay for pravastatin and lovastatin in human serum. Pharm Res 6: S237
12. Pentikäinen PJ, Saraheimo M, Schwartz JI, Amin RD, Schwartz MS, Brunner-Ferber F, Rogers JD (1992) Comparative pharmacokinetics of lovastatin, simvastatin and pravastatin in humans. J Clin Pharmacol 32: 136–140
13. Allenmark S, Edebo A, Lindgren K (1990) Determination of itraconazole in serum with high-performance liquid chromatography and fluorescence detection. J Chromatogr 532: 203–206
14. Wang RW, Kari PH, Lu AYH, Thomas PE, Guengerich FP, Vyas KP (1991) Biotransformation of lovastatin. IV. Identification of cytochrome P450 3A proteins as the major enzymes responsible for the oxidative metabolism of lovastatin in rat and human liver microsomes. Arch Biochem Biophys 290: 355–361
15. Vickers S, Duncan CA, Vyas KP, Kari PH, Arison B, Prakash SR, Ramjit HG, Pitzenberger SM, Stokker G, Duggan DE (1990) In vitro and in vivo biotransformation of simvastatin, an inhibitor of HMG CoA reductase. Drug Metab Dispos 18: 476–483
16. Lea AP, McTavish D (1997) Atorvastatin. A review of its pharmacology and therapeutic potential in the management of hyperlipidaemias. Drugs 53: 828–847
17. Kantola T, Kivistö KT, Neuvonen PJ (1998) Effect of itraconazole on the pharmacokinetics of atorvastatin. Clin Pharmacol Ther 64: 58–65
18. Bertz RJ, Granneman GR (1997) Use of in vitro and in vivo data to estimate the likelihood of metabolic pharmacokinetic interactions. Clin Pharmacokinet 32: 210–258
19. Transon C, Leemann T, Vogt N, Dayer P (1995) In vivo inhibition profile of cytochrome $P450_{TB}$ (CYP2C9) by (±)-fluvastatin. Clin Pharmacol Ther 58: 412–417
20. Haria M, McTavish D (1997) Pravastatin. A reappraisal of its pharmacological properties and clinical effectiveness in the management of coronary heart disease. Drugs 53: 299–336
21. Kivistö KT, Kantola T, Neuvonen PJ (1998) Different effects of itraconazole on the pharmacokinetics of fluvastatin and lovastatin. Br J Clin Pharmacol 46: 49–53
22. Mück W, Ochmann K, Rohde G, Unger S, Kuhlmann J (1998) Influence of erythromycin pre- and co-treatment on single-dose pharmacokinetics of the HMG-CoA reductase inhibitor cerivastatin. Eur J Clin Pharmacol 53: 469–473

REVIEWS OF THERAPEUTICS

Update: Clinically Significant Cytochrome P-450 Drug Interactions

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Recent technologies have resulted in an explosion of information concerning the cytochrome P-450 isoenzymes and increased awareness of life-threatening interactions with such commonly prescribed drugs as cisapride and some antihistamines. Knowledge of the substrates, inhibitors, and inducers of these enzymes assists in predicting clinically significant drug interactions. In addition to inhibition and induction, microsomal drug metabolism is affected by genetic polymorphisms, age, nutrition, hepatic disease, and endogenous chemicals. Of the more than 30 human isoenzymes identified to date, the major ones responsible for drug metabolism include CYP3A4, CYP2D6, CYP1A2, and the CYP2C subfamily.
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OUTLINE

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Nomenclature

The cytochrome P (CYP)-450 isoenzymes are a group of heme-containing enzymes embedded primarily in the lipid bilayer of the endoplasmic reticulum of hepatocytes (Figure 1).[1] These metabolic enzymes are also present in high concentrations in enterocytes of the small intestine, with smaller quantities in extrahepatic tissues (kidneys, lungs, brain). They are involved in oxidative metabolism (phase I) of a number of different drug classes as well as endogenous substances such as steroid hormones, fatty acids, and prostaglandins.[2-4] The nomenclature first suggested by Nebert et al in 1987 and widely used today employs a three-tier classification consisting of the family (> 36% homology in amino acid sequence), subfamily (77% homology), and individual gene (e.g., CYP3A4).[3-5]

Knowledge of the substrates, inhibitors, and inducers of CYP-450 isoenzymes assists in predicting clinically significant drug interactions. It is also important to recognize that genetic polymorphism in the functional expression of some CYP-450 isoenzymes, such as CYP2D6, contributes to marked interpatient variability in drug metabolism, leading to poor metabolizers (PMs) and extensive metabolizers (EMs).[2, 3, 6] In addition to genetic influences, microsomal drug metabolism is affected by age, nutrition, stress, hepatic disease, hormones, and other endogenous chemicals.[1] Although more than 30 human CYP-450 isoenzymes have been identified to date, the major ones responsible for drug metabolism are CYP3A4, CYP2D6, CYP1A2, and the CYP2C subfamily.

Substrates, Inhibition, and Induction

Some drugs may be metabolized by more than one isoenzyme. For example, the pharmacologically active enantiomer S-warfarin is metabolized by the CYP2C9 enzyme, whereas R-warfarin is metabolized by the CYP3A4 and CYP1A2 systems.[7, 8] Therefore, when one enzyme system is inhibited or induced by an interacting drug, a clinically significant interaction may or may not occur. Another example is tricyclic antidepressants, which are metabolized by CYP2D6, CYP1A2, and CYP3A4. Inhibition or genetic absence of one isoenzyme can lead to compensation through the secondary isoenzyme pathway. Similar to warfarin, oxidative metabolism can be preserved, and a clinically significant interaction may or may not occur.[9]

In addition, a drug may inhibit or induce the activity of a specific isoenzyme even though it is not a substrate at that particular site. For example, quinidine is metabolized by the CYP3A4 enzyme, but it is a potent inhibitor of CYP2D6.[2, 10]

Inhibition

Inhibition most often occurs as a result of competitive binding at the enzyme's binding site. Competitive inhibition depends on the affinity of the substrate for the enzyme being inhibited, the concentration of substrate required for inhibition, and the half-life of the inhibitor drug. The onset and offset of enzyme inhibition are dependent on the half-life and time to steady state of the inhibitor drug. For example, chloramphenicol (CYP2C9), acute ethanol ingestion, and cimetidine (CYP1A2) inhibit drug metabolism within 24 hours of a single dose, but amiodarone (CYP2C9) inhibitory interactions may not surface for months because of its long half-life.[11]

The time to maximum drug interaction (onset

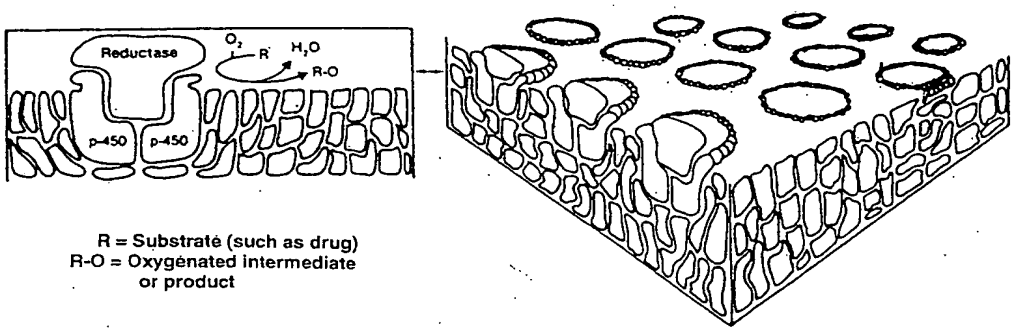

Figure 1. Cytochrome P-450 enzyme system. Reprinted by permission from Applied Pharmacokinetics: Principles of Therapeutic Drug Monitoring, third edition, edited by WE Evans, JJ Schentag, and WJ Jusko, published by Applied

and termination) is also dependent on the time required for the inhibited drug to reach a new steady state.[6,12] For example, with the cimetidine-theophylline interaction, maximum increases in theophylline concentrations are not seen for approximately 2 days, since this time is required for theophylline to reach a new steady state.[13] Another example is phenytoin. With its concentration-dependent half-life, steady-state changes in phenytoin serum concentration can take days to occur. Of interest, if the half-life of the inhibitor drug is shorter than that of the inhibited drug, less time is required to revert to a lower steady-state concentration after the inhibitor is stopped than is required to increase to a new steady-state concentration when the inhibitor is started. This is because the half-life of the affected drug is shorter after discontinuation of the inhibitor.[2-4]

A second and less common mechanism of inhibition is noncompetitive, which can occur as a result of inhibitor inactivation of the enzyme with normal substrate binding. The duration of this type of inhibition may be longer if new enzymes are synthesized after the inhibitor drug is discontinued.[1]

Another contributing factor to the significance of enzyme inhibition is the hepatic extraction ratio of the affected drug. In general, systemic clearance of low-extraction-ratio drugs is expected to be affected to a greater extent than that of high-extraction-ratio drugs. However, with high-extraction-ratio drugs with significant first pass metabolism, it is well known that significant changes in oral absorption can occur in the presence of inhibitory drugs.[1]

Induction

Enzyme induction, first recognized in the 1940s, occurs when hepatic blood flow is increased or the synthesis of more CYP-450 enzymes is stimulated. In animal models, phenobarbital increases liver weight in a dose-dependent manner. In humans, liver biopsies in patients taking anticonvulsants resulted in up to 52% larger absolute liver size.[14] Like inhibitors, inducers tend to be lipophilic, and the time course of the interaction is dependent on the half-life of the inducer. For example, rifampin's short half-life results in enzyme induction (CYP3A4, CYP2C) apparent within 24 hours, whereas phenobarbital's longer half-life of 3–5 days requires approximately 1 week for induction (CYP3A4, CYP1A2, CYP2C) to become apparent. Both of these agents interact with warfarin metabolism. Whereas rifampin's effects occur within 4 days, phenobarbital's effects take 14–22 days to occur.[15,16]

A complicating factor is that the time course of induction is also dependent on the time required for enzyme degradation and new enzyme production. In other words, the rate-limiting factor may be the half-life of CYP450 enzyme turnover, which ranges from 1–6 days.[17] Since rifampin is eliminated more rapidly than the excess cytochrome enzymes, the rate-limiting factor in the duration of the interaction would be enzyme turnover. With phenobarbital, accumulation and elimination would be the rate-limiting factor in the onset and offset of induction.[17]

Enzyme induction is also influenced by age and liver disease. The ability to induce drug metabolism may decrease with age, as evidenced by reports that drug metabolism in elderly subjects (> 60 yrs) is not influenced by polycyclic aromatic hydrocarbons (PAH) in cigarette smoke, as it is in younger subjects.[1,18] Also, patients with cirrhosis or hepatitis may be less susceptible to enzyme induction.[1]

CYP3A4 Isoenzyme

The CYP3A4 isoenzyme is responsible for the metabolism of the widest range of drugs and endogenous compounds in humans. It accounts for 60% of cytochrome enzymes in the liver and 70% of those in enterocytes found in the gut wall responsible for first-pass metabolism.[2,3,17] No evidence to date suggests the 3A4 isoenzyme exhibits genetic polymorphism.[19] Common substrates, inhibitors, and inducers of CYP3A4 are listed in Table 1.[2,3,9,10,16,17,19-117]

In recent years there has been an explosion of discussion about the 3A4 system because of life-threatening arrhythmic side effects that can occur as a result of enzyme inhibition and accumulation of the nonsedating antihistamines terfenadine and astemizole[20-25] and cisapride.[26-30]

Significant Inhibitory Interactions: Antihistamines

Terfenadine has been removed from the market because of its serious cardiovascular drug interactions. Its active carboxy metabolite, fexofenadine, is available and devoid of the fatal drug interactions.[31] Astemizole undergoes extensive first-pass metabolism to active metabolites, and, like terfenadine, the parent compound is the cardiotoxic entity.[32] In many cases, drug interactions with terfenadine have

Table 1. Cytochrome 3A4 Isoenzyme: Substrates, Inducers, and Inhibitors[2, 3, 9, 10, 16, 17, 19–117]

Substrates			
Alfentanil	Diazepam (minor)	Ketoconazole	Quinine
Alprazolam	Diltiazem	Lansoprazole (minor)	Rifampin
Amitriptyline (minor)	Disopyramide	Lidocaine	Ritonavir
Amlodipine	Donepezil	Losartan	Saquinavir
Astemizole	Doxorubicin	Lovastatin	Sertraline
Atorvastatin	Dronabinol	Mibefradil	Tacrolimus
Busulfan	Erythromycin	Miconazole	Tamoxifen
Cannabinoids	Estrogens, oral contraceptives	Midazolam	Temazepam
Carbamazepine	Ethosuximide	Navelbine	Terfenadine
Cisapride	Etoposide	Nefazodone	Testosterone
Clindamycin	Felodipine	Nelfinavir	Triazolam
Clomipramine	Fentanyl	Nicardipine	Verapamil
Clonazepam	Fexofenadine	Nifedipine	Vinblastine
Cocaine	Ifosfamide	Nimodipine	Vincristine
Cyclobenzaprine (demethylation)	Imipramine	Nisoldipine	R-warfarin
Cyclophosphamide	Indinavir	Ondansetron	Zileuton
Cyclosporine	Isradipine	Paclitaxel	
Dapsone		Pravastatin	
Dexamethasone		Prednisone	
Dextromethorphan		Quinidine	

Inhibitors		Inducers
Amiodarone	Metronidazole	Carbamazepine
Cannabinoids	Mibefradil	Dexamethasone
Clarithromycin	Miconazole	Ethosuximide
Erythromycin	Nefazodone	Phenobarbital
Fluconazole	Nelfinavir	Phenytoin
Fluoxetine	Norfloxacin	Primidone
Fluvoxamine	Quinine	Rifabutin
Grapefruit juice	Ritonavir	Rifampin
Indinavir	Saquinavir	Troglitazone
Itraconazole	Sertraline	
Ketoconazole	Troleandomycin	
Omeprazole (slight)	Zafirlukast	

been extrapolated to astemizole.[31] Terfenadine, available since 1985, was first reported in 1990 to cause QT prolongation and torsades de pointes when given together with ketoconazole.[23] A prospective study of six healthy volunteers given the combination noted increased parent terfenadine concentrations and QT prolongation (mean 82-msec increase).[22]

In vitro, the ability of itraconazole, an antifungal similar to ketoconazole, to inhibit the 3A4 system is 10 times less potent than that of ketoconazole, but inhibitory differences in vivo are less impressive.[33] Fluconazole also inhibits 3A4 in vitro, but did not increase parent terfenadine concentrations or cause arrhythmias at dosages of 200 mg/day.[32] However, dosages above 200 mg/day caused QT prolongation in subsets of patients.[34]

The antifungal agents itraconazole, ketoconazole, fluconazole, and intravenous miconazole should not be coadministered with astemizole due to the serious nature of potential drug interactions, although single doses of fluconazole for candidiasis are not likely to present a problem. The new antifungal terbinafine does not appear to inhibit the 3A4 system and is an alternative for the treatment of onychomycosis.[118]

Erythromycin alone can cause QT prolongation,[119] and when combined with terfenadine does so as well (mean 10-msec increase) but to a lesser degree than when given with antifungal agents.[20] This effect was also reported with clarithromycin[31] but not with azithromycin or dirithromycin, which may be alternatives for patients receiving astemizole.[35–37]

In vitro evidence exists for inhibition of the 3A4 isoenzyme by the antidepressant drugs fluvoxamine, fluoxetine, nefazodone, and sertraline. In addition, plasma concentrations of drugs metabolized by 3A4 such as carbamazepine and some benzodiazepines increased when given concomitantly with these four agents.[9, 19, 39, 40] To

date, there are no in vitro data or case reports involving paroxetine in the inhibition of 3A4.[19]

Data on significant drug interactions with these antidepressants and antihistamines is less clear than with antifungals and macrolides. Fluoxetine caused arrhythmias in patients concomitantly receiving terfenadine.[41, 42] Another concern with fluoxetine is the long half-life of the parent compound (4–6 days) and its active metabolite norfluoxetine (4–16 days). The consequences of an interaction may be minimized by delaying administration of astemizole for 2–4 weeks after discontinuing fluoxetine.[43] Prescribing information for both fluvoxamine and nefazodone lists concomitant administration with astemizole as a contraindication,[44–46] and sertraline information warns against concomitant administration with astemizole.[47] Thus, fluoxetine, fluvoxamine, nefazodone, and sertraline should be administered cautiously, if at all, to patients taking astemizole (Table 2).[2, 3, 7–11, 14–118] In patients receiving astemizole, alternatives for the treatment of depression are paroxetine[19] and venlafaxine.[48] The tricyclic antidepressants should also be prescribed cautiously since they can cause arrhythmias.[9]

Fresh or frozen grapefruit juice inhibits CYP3A4 enzymes found in enterocytes. The inhibitory substance was once thought to be naringenin, a human metabolite of nargingin.[49–51] However, the primary substance responsible for inhibition was identified in vitro to be a furanocoumarin compound widely found in nature, 6,7-dihydroxybergamottin. This inhibitory substance is less potent than ketoconazole but considerably more active than cimetidine. Lack of 6,7-dihydroxybergamottin in orange juice probably accounts for the absence of cytochrome inhibitory effects.[52–54] Inhibition of terfenadine metabolism with quantifiable levels of the terfenadine parent compound, an increase in area under the curve (AUC) of 55%, and a mean QT prolongation of 14 msec were reported in patients ingesting grapefruit juice 240 ml concomitantly with terfenadine 60 mg twice/day.[55, 56] Other studies reported similar pharmacokinetic changes but associated with no electrocardiographic changes.[57]

Recently the calcium channel blocker, mibefradil, has been shown to inhibit both CYP3A4 and CYP2D6 and cause syncope in some patients taking β-blockers. Because mibefradil could theoretically increase plasma concentrations of astemizole, its concurrent use should be avoided.[38]

Another cytochrome 3A4 inhibitor is quinine. At dosages greater than 430 mg/day, quinine is contraindicated with astemizole since the combination may result in QT prolongation.[58] It is prudent to limit the use of quinine and tonic water in patients receiving astemizole.

In vitro, the protease inhibitors saquinavir, ritonavir, indinavir, and nelfinavir inhibit cytochrome 3A4.[59] To date, neither pharmacokinetic studies nor in vivo drug-drug interaction studies have been conducted for these agents with antihistamines. Prescribing information for ritonavir[60] lists concomitant administration with astemizole as a contraindication, and prescribing information for saquinavir,[61] indinavir,[62] and nelfinavir[63] issue precautions regarding concomitant astemizole administration because of the potential for life-threatening cardiotoxic interactions.

Other recommendations to minimize the risk of cardiotoxic drug interactions include avoiding astemizole dosages greater than 10 mg/day, prescribing alternative agents (Table 2), prescribing astemizole cautiously in patients with cardiac conditions that predispose them to QT prolongation, administering the drug cautiously in patients taking other agents that can prolong the QT interval (e.g., type Ia or III antiarrhythmics; some psychotropics such as haloperidol, droperidol, tricyclic antidepressants), and administering astemizole cautiously in patients with hepatic disease.

Significant Inhibitory Interactions: Cisapride

The fact that cisapride can cause tachycardia, palpitations, and extrasystoles was first observed in a review of records of over 13,000 patients receiving the agent.[28] Postulations about the cause of tachycardia include activation of serotonin-4 receptors on the myocardium[63] and prolonged atrioventricular conduction due to its structural similarity to procainamide.[30] The first report of an arrhythmic drug interaction with cisapride was with erythromycin (for 2 days only) with dosages of cisapride that were rapidly escalated to 40 mg every 6 hours. The patient developed a QT interval of 550 msec from a normal baseline with progression to polymorphic nonsustained ventricular tachycardia. The QT interval returned to normal after the cisapride dosage was decreased to 5 mg every 6 hours.[29]

Janssen Pharmaceutica continues to receive numerous reports of torsades de pointes, prolonged QT intervals, and deaths.[27] Over 50% of these patients were concomitantly receiving ketoconazole, itraconazole, or fluconazole, and erythromycin,

Table 2. 3A4 Clinically Significant Drug Interactions[2–3, 7–11, 14–118]

Substrate	Inhibitor, Inducer, Competing Substrate	Management	Alternatives
Antiarrhythmics			
Disopyramide Lidocaine (less likely) Quinidine	Carbamazepine (ind) Phenobarbital (ind) Phenytoin (ind)	May need initial dosage increase; monitor SC&E.	Gabapentin Lamotrigine Topiramate Valproate
Disopyramide Lidocaine	Indinavir (inh) Nelfinavir (inh) Ritonavir (inh) Saquinavir (inh)	Decrease initial dosage 50%; monitor SC&E.	
Disopyramide Quinidine	Rifampin (ind)	Need initial dosage increase; monitor SC&E.	
	Clarithromycin (inh) Erythromycin (inh) Troleandomycin (inh)	Reports of increased quinidine levels; monitor QT interval.	Azithromycin Dirithromycin
Lidocaine	Cimetidine (inh)	Reports of toxicity not consistent; up to 30% increase in serum conc; monitor SC&E.	Famotidine Nizatidine Ranitidine
Quinidine	Cimetidine (inh)	Conflicting data; up to 50% increase in serum conc reported; monitor SC&E.	Famotidine Nizatidine Ranitidine
	Phenobarbital (ind) Phenytoin (ind) Rifampin (ind)	Monitor concentrations more carefully.	Gabapentin Lamotrigine Topiramate Valproate
	Metronidazole	3-fold increase in trough conc; monitor SC&E.	
	Amiodarone (inh)	Decrease dosage 30–50% on initiation; monitor QT interval.	
	Ketoconazole (inh) Itraconazole (inh) Fluconazole (inh) Miconazole i.v. (inh)	30-fold increase in serum conc after 7 days, monitor QRS.	
Anticoagulants			
R-warfarin	Cisapride (inh)	One report of increased INR.	Metoclopramide
	Amiodarone (inh)	Delayed interaction; decrease dosage 25% on initiation.	
	Fluconazole (inh) Itraconazole (inh) Ketoconazole (inh)	May cause 2-3-fold increase in INR; monitor INR more carefully on starting or stopping; may also be 1A2 mediated.	
	Erythromycin (inh) ?Clarithromycin (inh)	Seen within 7 days; monitor INR daily.	Azithromycin Dirithromycin
	Omeprazole (inh)	Effects appear after a few days; dose related; monitor INR more carefully.	Lansoprazole
Anticonvulsants			
Carbamazepine	Erythromycin (inh) Clarithromycin (inh)	Decrease dosage by 25%; seen within 24 hrs.	Azithromycin Dirithromycin
	Fluoxetine (inh) Fluvoxamine (inh) Sertraline (inh)	Anecdotal reports of increased conc with blurred vision, tremor in some patients; monitor SC&E.	
	Isoniazid (inh)	Can cause toxicity; more pronounced in slow acetylators.	
Carbamazepine Ethosuximide	Indinavir (inh) Nelfinavir (inh) Ritonavir (inh) Saquinavir (inh)	Decrease initial dosage 50%; monitor serum conc.	

Table 2. 3A4 Clinically Significant Drug Interactions[2, 3, 7–11, 14–118] (continued)

Substrate	Inhibitor, Inducer, Competing Substrate	Management	Alternatives
Carbamazepine Ethosuximide	Rifampin (ind)	Monitor serum conc; may need initial dosage increase.	
Antifungal agents			
Itraconazole Ketoconazole	Rifampin (ind)	Consider dosage increase; poor clinical response reported; fluconazole less affected.	Fluconazole
	Carbamazepine (ind) Phenobarbital (ind) Phenytoin (ind)	Poor clinical response noted.	Fluconazole Gabapentin Lamotrigine Topiramate Valproate
Antidepressants			
Nefazodone Sertraline Trazodone Desipramine	Indinavir (inh) Nelfinavir (inh) Ritonavir (inh) Saquinavir (inh)	Decrease initial dosage 50%; monitor for SE.	
Antiemetics			
Dronabinol Ondansetron	Indinavir (inh) Nelfinavir (inh) Ritonavir (inh) Saquinavir (inh)	Decrease initial dosage 50%; monitor for SE.	
Antifungal agents			
Itraconazole Ketoconazole	Phenobarbital (ind) Phenytoin (ind) Rifampin (ind)	Therapeutic failures reported; fluconazole not as affected.	Fluconazole
Antihistamines and cisapride			
Astemizole Cisapride	Clarithromycin (inh) Erythromycin (inh) Troleandomycin (inh)	Avoid these combinations.	Cetirizine[a] Clemastine[a] Loratadine[a] Metoclopramide[a] Azithromycin Dirithromycin
	Fluconazole (inh) Itraconazole (inh) Ketoconazole (inh) Miconazole i.v. (inh)	Avoid use.	Terbinafine for onychomycosis
	Fluoxetine (inh) Fluvoxamine (inh) Nefazodone (inh) Sertraline (inh)	Avoid combinations.	Paroxetine Venlafaxine
	Grapefruit juice (inh)	Avoid > 200 ml/day.	Orange juice, other juices
	Quinine, tonic water (inh)	Avoid > 430 mg/day.	
	Indinavir (inh) Nelfinavir (inh) Ritonavir (inh) Saquinavir (inh)	Avoid combinations.	Other antiretrovirals
	Mibefradil (inh)		Other CCBs
	Zafirlukast (inh) Zileuton (inh)	Caution with combinations; 35% increase in terfenadine conc reported with zileuton.	Other asthma regimens
Loratadine	Indinavir (inh) Ritonavir (inh) Saquinavir (inh)	Decrease initial loratadine dosage 50%; therapeutic monitoring; no cardiac side effects reported.	
Cisapride	Metronidazole (inh)	Avoid combination.	Metoclopramide, other antibiotics

Table 2. 3A4 Clinically Significant Drug Interactions[2,3,7–11,14–118] (continued)

Substrate	Inhibitor, Inducer, Competing Substrate	Management	Alternatives
Benzodiazepines			
Alprazolam Midazolam Triazolam	Fluoxetine (inh) Fluvoxamine (inh) Nefazodone (inh) ?Grapefruit juice	Decrease initial alprazolam dosage 50% and triazolam dosage 75%; monitor for oversedation.	Temazepam
	Indinavir (inh) Nelfinavir (inh) Ritonavir (inh) Saquinavir (inh)	Delayed interaction; adjust dosage; monitor for SE.	Temazepam Other antiretrovirals
Midazolam Triazolam ?Other benzodiazepines	Rifampin (ind)	Hypnotic effects substantially diminished; monitor and give higher initial dosages of benzodiazepine.	
Midazolam Triazolam	Erythromycin (inh)	Decrease initial triazolam dosage 50%; monitor for oversedation.	Azithromycin Dirithromycin
Triazolam Others	Itraconazole (inh) Ketoconazole (inh) Fluconazole (inh)	27-fold increase in conc with sedation, decreased psychomotor abilities.	
Midazolam Triazolam	Cimetidine (inh)	Some CNS effects documented.	Famotidine Nizatidine Ranitidine
Calcium channel blockers			
Amlodipine Felodipine Isradipine Mibefradil	Grapefruit juice (inh) (200 ml/day)	Decrease initial CCB dosage 50%; monitor for dizziness, headache, peripheral edema, hypotension.	Orange juice, other juices
Nicardipine Nifedipine Nimodipine Nisoldipine Verapamil	Erythromycin (inh)	Decrease initial CCB dosage 50%; monitor for SE.	Azithromycin Dirithromycin
	Itraconazole (inh) Ketoconazole (inh)	Decrease initial CCB dosage 50%; monitor for SE.	?Fluconazole
	Indinavir (inh) Nelfinavir (inh) Ritonavir (inh) Saquinavir (inh)		Other antiretrovirals
	Rifampin (ind) Rifabutin (ind)	May need initial dosage increase; monitor clinical effects; best documented with diltiazem, verapamil, nifedipine.	
Nifedipine Others	Phenobarbital (ind)	Theoretical; best documented with nifedipine and phenobarbital.	
Nifedipine Verapamil	Cimetidine (inh)	Conflicting results; some reports of decreased BP, HR.	Famotidine Nizatidine Ranitidine
Chemotherapeutic agents			
Busulfan	Itraconazole (inh)	Report of increased serum conc in BMT pts.	Fluconazole
Doxorubicin	Cyclosporine (inh) Paclitaxel (inh)	Elevated concentrations; more nausea and vomiting.	
Etoposide	Phenobarbital (ind) Phenytoin (ind)	170% increase in clearance.	
	Cyclosporine (inh)	2-fold increase in half-life.	

Table 2. 3A4 Clinically Significant Drug Interactions[2,3,7–11,14–118] (continued)

Substrate	Inhibitor, Inducer, Competing Substrate	Management	Alternatives
Etoposide Paclitaxel Tamoxifen Vinblastine Vincristine	Indinavir (inh) Nelfinavir (inh) Ritonavir (inh) Saquinavir (inh)	Decrease initial chemotherapy dosage 50%.	
Tamoxifen	Erythromycin (inh) Cyclosporine (inh) Nifedipine (inh) Diltiazem (inh)		
Vinblastine	Doxorubicin (inh) Etoposide (inh) Ketoconazole (inh) Erythromycin (inh)		
Vincristine	Nifedipine (inh)		
Estrogens, corticosteroids			
Oral contraceptives	Benzodiazepines (ind)	Induce or compete with contraceptives; use alternative contraception for short courses; for long courses use higher dosages or medroxyprogesterone acetate.	Medroxyprogesterone acetate
Oral contraceptives Corticosteroids	Rifampin (ind) Rifabutin (ind)	Use alternative contraception or increase dosage to 50 µg estradiol.	
	Carbamazepine (ind) Ethosuximide (ind) Phenobarbital (ind) Phenytoin (ind) Primidone (ind)	40% reduction in serum levels; monitor for breakthrough bleeding; alternative contraception (e.g., medroxyprogesterone) desirable.	Gabapentin Lamotrigine Topiramate Valproate
Oral contraceptives	Troglitazone (ind)	Reduces concentrations by 30%; use alternative contraception.	
Methylprednisolone Prednisolone ?Other corticosteroids	Ketoconazole (inh)	AUC increased; significance unknown.	
HMG-CoA reductase inhibitors			
Lovastatin Atorvastatin Fluvastatin Pravastatin Simvastatin	Erythromycin (inh) Clarithromycin (inh) Troleandomycin (inh)	Monitor for myopathy; most common with lovastatin.	Azithromycin Dirithromycin
	Itraconazole (inh) Cyclosporine (inh)	Monitor for myopathy.	
	Indinavir (inh) Nelfinavir (inh) Ritonavir (inh) Saquinavir (inh)	Decrease initial dosage 50%; therapeutic monitoring.	Other antiretrovirals
Immunosuppressants			
Cyclosporine Tacrolimus	Indinavir (inh) Nelfinavir (inh) Ritonavir (inh) Saquinavir (inh)	Decrease initial dosage 50%; therapeutic monitoring.	Other antiretrovirals
	Norfloxacin (inh)	Seen in pediatric transplant patients.	Ciprofloxacin
	Rifampin (ind)	May need initial dosage increase; monitor SC&E.	
	Amiodarone (inh)	Decreases clearance by 50%; monitor SC&E.	

Table 2. 3A4 Clinically Significant Drug Interactions[2, 3, 7–11, 14–118] (continued)

Substrate	Inhibitor, Inducer, Competing Substrate	Management	Alternatives
Cyclosporine Tacrolimus	Erythromycin (inh) Clarithromycin (inh) Troleandomycin (inh) Roxithromycin (inh)	Avoid combination or reduce dosage by 50%; can see within 2 d; monitor trough conc 2–3 x/wk.	Azithromycin, Dirithromycin
	Fluconazole (inh) Itraconazole (inh) Ketoconazole (inh)	Consider 50% dosage reduction when starting azole; monitor trough conc more carefully.	Ketoconazole 100–200 mg often given to improve F
	Mibefradil (inh) Nicardipine (inh) Nifedipine (inh) Diltiazem (inh) Verapamil (inh)	Monitor trough conc more carefully.	Diltiazem often given to improve F Amlodipine Isradipine Nitrendipine
	Methylprednisolone (inh)	Competitive metabolism; monitor trough conc.	
	Carbamazepine (ind) Phenobarbital (ind) Phenytoin (ind)		Gabapentin Lamotrigine Topiramate Valproate
	Oral contraceptives (inh)	Not well documented; may need dosage decrease; monitor trough conc.	
Macrolides			
Clarithromycin	Indinavir (inh) Nelfinavir (inh) Ritonavir (inh) Saquinavir (inh)	AUC increases by 77% with ritonavir and 53% with indinavir; decrease clarithromycin dosage by 50% for Cl_{cr} 30–60 ml/min and 75% for Cl_{cr} < 30 ml/min.	
	Rifampin (ind) Rifabutin (ind)	Will decrease serum conc by 120% with rifampin and 50% with rifabutin; clinical significance unknown.	
Erythromycin	Ritonavir (inh)	Decrease initial dosage 50% (based on in vitro data).	
Miscellaneous			
Rifabutin	Clarithromycin (inh)	Increase serum conc; increased risk of icterus and uveitis; monitor for ocular SE.	
	Fluconazole (inh)	Increases AUC by 80%; increased risk of icterus and uveitis; monitor for ocular SE.	
	Indinavir (inh) Nelfinavir (inh)	204% increase in AUC; decrease initial rifabutin dosage by half.	
	Ritonavir (inh)	4-fold increase in AUC; increased risk of SE; combination contraindicated.	Alternative MAC prophylaxis
Cyclobenzaprine	Fluoxetine (inh)	Observe for QT prolongation.	
Narcotic analgesics			
Alfentanil	Erythromycin (inh)	Monitor for oversedation; marked increase in AUC of opiate.	Azithromycin
Alfentanil Fentanyl	Indinavir (inh) Nelfinavir (inh) Ritonavir (inh) Saquinavir (inh)	Decrease initial dosage 50%; monitor for oversedation.	
	Rifampin (ind)	Increase initial dosage; monitor for withdrawal.	
Fentanyl	Cimetidine (inh)	Reports of doubling in terminal half-life; monitor for SE.	Famotidine Nizatidine Ranitidine

Table 2. 3A4 Clinically Significant Drug Interactions[2–3, 7–11, 14–118] (continued)

Substrate	Inhibitor, Inducer, Competing Substrate	Management	Alternatives
Protease inhibitors Indinavir Nelfinavir Ritonavir Saquinavir	Rifabutin (ind) Rifampin (ind)	40% decrease in saquinavir AUC with rifabutin and 80% decrease with rifampin; rifampin decreases ritonavir AUC 35%; may need dosage increase; see specific MMWR guidelines.	Nucleoside or nonnucleoside combinations
	Carbamazepine (ind) Phenobarbital (ind) Phenytoin (ind) Tobacco (ind)	Use cautiously.	Nucleoside or nonnucleoside combinations Gabapentin Lamotrigine Topiramate Valproate
Indinavir	Clarithromycin (inh) Erythromycin (inh) Troleandomycin (inh)	Increases AUC 30%.	Azithromycin Dirithromycin
Indinavir Saquinavir	Ketoconazole (inh) Fluconazole (inh)	Ketoconazole increases AUC by 150%; with ketoconazole, decrease initial indinavir dosage to 600 mg q8h; decrease saquinavir dosage if ketoconazole dosage is > 200 mg/day.	
Ritonavir	Smoking (ind)	18% decrease in AUC; no specific recommendations at present.	
Saquinavir	Ritonavir (inh) Other protease inhibitors?	Increases conc by 18-fold to improve saquinavir absorption.	

SC&E = serum concentration and effects; INR = international normalized ratio; SE = side effects; CCB = calcium channel blockers; BP = blood pressure; HR = heart rate; BMT = bone marrow transplantation; F = bioavailability; Cl_{cr} = creatinine clearance; MAC = *Mycobacterium avium intracellulare* complex; AUC = area under the concentration-time curve.
*Alternative antihistamines and prokinetic agents in all interactions listed.

clarithromycin, or metronidazole. Risk factors for arrhythmia were identified as history of coronary disease and arrhythmia, renal insufficiency, electrolyte imbalance, and long-term use of agents associated with arrhythmia or prolonged QT intervals such amiodarone and phenothiazines.[27]

Three reports in our institution in 1995–1996 involved fluconazole or erythromycin interactions with cisapride. Two patients were receiving both fluconazole 100 and 400 mg/day and cisapride 20 mg every 6 hours, had no known cardiac disease, developed ventricular fibrillation, and had resolution with no further arrhythmias after cisapride was discontinued. One of these patients initially had recurrence of sustained ventricular tachycardia after lidocaine was discontinued and before cisapride was discontinued. The third patient had a history of cardiac disease and was receiving erythromycin 500 mg intravenously every 6 hours plus oral cisapride 10 mg every 12 hours. His QT interval increased from 352 msec at baseline to 440 msec, and he died from ventricular fibrillation-cardiac arrest. The drug interaction was postulated as a potential culprit.

Similar to antihistamines, only in vitro data about enzyme inhibition, and no actual patient-specific data, are the bases for contraindications and warnings against concomitant cisapride with other drugs such as fluvoxamine, mibefradil, nefazodone, sertraline, ritonavir, saquinavir, and indinavir.[38, 44–46, 60–62] It would also seem prudent to avoid administering fluoxetine, quinine, and grapefruit juice with cisapride because of the fatal consequences of potential interactions, and because similar interactions were documented with terfenadine. As is the case with astemizole, steps to minimize the cisapride drug interactions include avoiding cisapride dosages greater than 20 mg every 6 hours and exercising caution in patients with hepatic disease or other risk factors for QT prolongation.

Hypoprothrombinemic effects of warfarin were reported when given in combination with

cisapride. The patient's international normalization ratio (INR) at baseline was 2.2–2.5 and increased to 10.7, first noted 3 weeks after the patient began cisapride 10 mg 4 times/day. Competitive binding or inhibition by cisapride was postulated.[69] The clinical significance of this interaction remains to be established.

Significant Inhibitory Interactions: R-Warfarin

Fluconazole, itraconazole, and ketoconazole reportedly increase the anticoagulant effects of warfarin. Two-fold (fluconazole) and 3-fold (ketoconazole) increases in prothrombin time have been reported. Even low dosages of fluconazole 100 mg/day for 7 days were implicated to reduce the clearance of both isomers of warfarin.[7, 8, 33]

Numerous reports describe enhancement of the hypoprothrombinemic effects of warfarin when given in combination with erythromycin. Prothrombin times increased up to 2-fold after 7 days of therapy, but there are few reports of bleeding complications. The clinical relevance of this interaction probably depends on many patient factors including age, rate of warfarin clearance, concurrent drug therapy, and ability to shunt to noninhibited pathways.[33] The interaction has not been observed with azithromycin, but like erythromycin, caution is advised with concurrent clarithromycin therapy.[33, 36]

Omeprazole has a benzimidazole moiety similar to the imidazole ring of cimetidine and has been studied for inhibitory drug interactions. It inhibits the metabolism of R-warfarin, and this interaction is likely to be 3A4 mediated. The effects appear after omeprazole has been taken for a few days, seem to be dose related, and do not abate immediately on discontinuing the drug. Careful INR monitoring is recommended in patients receiving this combination. Lansoprazole does not alter the clearance of warfarin and may be an alternative treatment.[65–68]

Significant Inhibitory Interactions: Benzodiazepines and Narcotic Analgesics

Alfentanil, alprazolam, midazolam, temazepam, and triazolam are among the currently known substrates of cytochrome 3A4.[9, 19, 39, 40, 72] The benzodiazepines have fairly well documented interactions. Pharmacokinetic studies with alprazolam showed increased serum concentrations and prolonged half-life when given with the inhibitors fluoxetine and fluvoxamine.[19] Fluoxetine increased plasma concentrations of diazepam and alprazolam, but enhancement of psychomotor effects was not seen.[43]

Another antidepressant, nefazodone, increased alprazolam plasma concentrations 2-fold and potentiated alprazolam-induced psychomotor impairment and sedation.[71–74] Nefazodone also increased triazolam plasma concentrations and half-life by 1.7- and 3-fold, respectively.[71–73] Initial dosage reductions of alprazolam by 50% and triazolam by 75% should be made when adding nefazodone to existing therapy with these agents.[71–74]

Grapefruit juice 200 ml increased peak plasma concentrations of orally administered midazolam by 56% and AUC by 52%. The clinical importance of this increase is unknown.[49]

Temazepam, although metabolized by the 3A4 system, does not result in significant pharmacokinetic or pharmacodynamic interactions as assessed by psychomotor performance with inhibitors such as erythromycin and itraconazole.[76, 77] Lack of interaction potential with temazepam compared with midazolam, alprazolam, and triazolam may be due to different metabolic pathways and lack of significant first-pass metabolism of temazepam. Erythromycin reduced clearance of triazolam by 52% and decreased midazolam clearance enough to cause unconsciousness. If it is not possible to avoid these combinations, the benzodiazepine dosage should be decreased by 50% and the patient monitored carefully for respiratory depression and other signs of toxicity.[36] Inhibitors of 3A4 should therefore be administered with caution to patients taking alprazolam or triazolam and to those undergoing surgical procedures requiring midazolam as a component of anesthesia.

For the opioids, the only well-documented 3A4-mediated interaction is with alfentanil and erythromycin.[70, 79] Reports include prolonged respiratory depression associated with alfentanil in patients who were receiving erythromycin before surgery.[70] Administration of cimetidine with fentanyl doubles the latter's elimination half-life, thus potentially enhancing its pharmacologic effects and duration of action.[70]

Significant Induction Interactions: Benzodiazepines and Narcotic Analgesics

Rifampin can significantly impair the efficacy of some benzodiazepines. A 96% reduction in the AUC of midazolam was accompanied by nonexistent hypnotic effects when administered with rifampin to 10 healthy volunteers in a

double-blind crossover study.[78] Similar results were reported with triazolam and rifampin, with markedly reduced effects of triazolam based on psychomotor tests.[79]

Like benzodiazepines, it is well known that rifampin increases the rate of metabolism of many opioids and may induce withdrawal symptoms.[16, 70, 77]

Significant Inhibitory Interactions: Cardiovascular Drugs

Most calcium channel-blocking agents are dependent on the 3A4 isoenzyme system for metabolism. Other cardiovascular drugs metabolized by 3A4 are the hydroxymethylglutaryl-CoA (HMG-CoA) reductase inhibitors.[1, 17]

Grapefruit juice 200–250 ml given before drug administration increased the AUC of felodipine by 185%, with an average increase of 240%. Similar results occurred with nifedipine, nimodipine, and verapamil but not with diltiazem.[50, 55] Furthermore, this pharmacokinetic interaction was clinically significant, with lower diastolic blood pressure, higher heart rate, and more frequent vasodilation-related side effects with felodipine, nisoldipine (5-fold increase in AUC), and nifedipine.[55, 80, 81] This information supports cautioning patients about concomitant ingestion of grapefruit juice and calcium antagonists.

A patient taking oral felodipine 10 mg/day was given oral erythromycin 250 mg twice/day and developed flushing, ankle and leg edema, and tachycardia. When erythromycin was discontinued, felodipine levels were reduced from 6 to less than 2 nmol/L and symptoms resolved.[82] On average, it appears that erythromycin increases felodipine concentrations by 3-fold.[55] Other studies have demonstrated similar increases in felodipine concentrations when given with erythromycin.[83] Other reports documented substantial peripheral edema and/or elevated calcium antagonist serum concentrations during concurrent administration of itraconazole with felodipine, isradipine, or nifedipine.[84–86] An 8-fold increase in feldopine's AUC was seen when the agent was given with itraconazole 200 mg/day, and was associated with statistically significant changes in systolic and diastolic blood pressures and heart rate.[86]

If concurrent therapy of potent 3A4 inhibitors with calcium antagonists is required, the patient should be monitored for signs of toxicity and the dosage of calcium channel blocker decreased, if necessary.

As mentioned, HMG-CoA reductase inhibitors are metabolized by the 3A4 system and have dose-related toxic effects on skeletal muscle that may range from diffuse myalgia and myopathy to severe rhabdomyolysis. These effects are reported most frequently with lovastatin but have also been reported rarely with the other statins.[87, 88] The risk of rhabdomyolysis appears to be greatest when HMG-CoA reductase inhibitors are combined with 3A4 inhibitor drugs or agents that compete with 3A4 metabolism.[89–94] This interaction has been well described with cyclosporine, and less often with gemfibrozil and niacin.[89]

In a double-blind, crossover trial, itraconazole increased lovastatin peak concentrations by 20-fold in 12 healthy volunteers. Side effects were not reported in any subjects except one who experienced a 10-fold increase in creatine kinase.[90] Reports of severe rhabdomyolysis occurring after the addition of itraconazole to lovastatin and niacin therapy underscore the potential harm of these interactions. Myopathy also occurred after itraconazole was added to cyclosporine and simvastatin therapy in a transplant recipient.[91] Numerous authors described the development of rhabdomyolysis when lovastatin was combined with erythromycin. Myopathy is quickly reversible when the statin is discontinued.[92–94]

Concomitant administration of ritonavir and lovastatin increased the AUC of lovastatin by 3-fold. Little information is available about protease inhibitors, but careful dosing with statins is prudent.[58, 60–62] Giving statins with 3A4 inhibitors should be avoided or dosages of statins reduced to avoid the potential for rhabdomyolysis. Patients should be instructed to monitor for signs of myopathy such as muscular pain, tenderness, or weakness, and plasma creatine kinase should be measured if symptoms develop.

Significant Induction Interactions: Calcium Channel Blockers

In one study, enzyme induction with rifampin resulted in up to a 32-fold increase in verapamil clearance and a 25-fold decrease in verapamil bioavailability after oral administration.[95] The effect of oral verapamil on atrioventricular conduction was nearly abolished with rifampin administration, and the authors concluded that prehepatic metabolism of verapamil was induced by rifampin. Rifampin is expected to have a similar reaction with other calcium channel blockers.

Significant Interactions: Quinidine

Quinidine is known to be a cytochrome 2D6 inhibitor but is metabolized by the 3A4 system. Cytochrome 3A4 interactions that are well documented include those with cimetidine,[2, 13] phenytoin, phenobarbital,[2, 120] and rifampin.[2, 114] Of interest, metronidazole is a 3A4 inhibitor due to its interaction with cisapride,[27] and a potential interaction of quinidine with either metronidazole or ciprofloxacin was reported. A 3-fold difference in quinidine trough concentrations was noted, but with no changes in the patient's QT interval.[10] An interaction with metronidazole seems more likely since it is a known 3A4 inhibitor. Quinidine concentrations should be monitored and patients assessed for signs of toxicity in these instances.

Significant Inhibitory and Induction Reactions: Chemotherapeutic Agents

Little is known about pharmacokinetic interactions with chemotherapeutic agents, but it is likely that important interactions have not been identified. The 3A4 enzyme was important in the metabolism of several agents, including epipodophylotoxins, tamoxifen, ifosfamide, paclitaxel, and vinca alkaloids. Although beyond the scope of this review, it is interesting to note that 3A4 catalyzes the activation of the prodrug ifosfamide, raising the possibility that it could be activated in tumor tissues containing this enzyme. Cytochrome 3A4 substrates may also modulate multidrug resistance to cancer chemotherapy.[97]

Cyclosporine increased the AUC of doxorubicin by 55% and decreased doxorubicin clearance by 50%. The addition of cyclosporine also increased doxorubicin-induced nausea and vomiting. Similar myelosuppression was observed when comparing doxorubicin alone with doxorubicin (60% of the control dose) plus cyclosporine.[98] Similar pharmacokinetic results were obtained and a higher frequency of drug-related toxicity was observed in patients receiving cyclosporine.[99] Simultaneous administration of doxorubicin and paclitaxel also resulted in significantly elevated concentrations of doxorubicin, suggesting that paclitaxel may inhibit its metabolism.[100]

Ifosfamide is an alkylating agent that requires biotransformation to produce its pharmacologically active cytoxic compound. This activation by the 3A4 system also results in the formation of a therapeutically inactive but neurotoxic metabolite by *N*-dechlorethylation. Few studies have been done, but 3A4 inducers such as rifampin, carbamazepine, phenobarbital, and phenytoin are postulated to enhance efficacy and toxicity through 3A4 activation. In immunohistochemical studies, some patients with pulmonary carcinoma showed expression of the 3A4 enzyme, and studies are continuing to assess if this presence leads to local activation and a better response to ifosfamide.

In contrast, inhibitors and other substrates of 3A4, such as ketoconazole, itraconazole, diltiazem, verapamil, and cyclosporine, could possibly interfere with activation and efficacy of ifosfamide The clinical significance of inhibition is unknown at present.[97]

In vitro, vinblastine metabolism is inhibited by other anticancer drugs, including doxorubicin and etoposide, together with more familiar inhibitors, ketoconazole and erythromycin. Although not studied clinically, these interactions may alter the antitumor activity and/or toxicity of vinblastine.[101] Concomitant treatment with vincristine, another vinca compound, and nifedipine resulted in a 4-fold increase in vincristine's elimination half-life. Clinical studies are necessary to validate the pharmacokinetic data, but greater cytotoxicity could be anticipated.[102]

Busulfan is another chemotherapeutic agent that may be metabolized through the 3A4 system. A study in 13 bone marrow transplant recipients found an average 20% reduction in busulfan clearance in patients receiving itraconazole compared with those receiving either fluconazole or placebo. Itraconazole is known to be a more potent inhibitor of 3A4 than fluconazole.[34] The nature of this interaction has yet to be elucidated, but inhibition of oxidative metabolism may be a factor.[103]

Etoposide is significantly affected when administered concurrently with inducers such phenobarbital and phenytoin, with a mean 170% increase in clearance reported with these drugs. On the other hand, concurrent administration with cyclosporine resulted in an 80% increase in AUC and a 2-fold increase in etoposide half-life.[97] In vitro, tamoxifen metabolism is inhibited by erythromycin, cyclosporine, nifedipine, and diltiazem. No clinical data are available, but interactions are likely to occur and should be investigated.[104]

Few clinical data exist to make sound conclusions regarding interactions with chemotherapeutic agents. However, significant interactions with cytochrome 3A4 inhibitors or inducers are likely to become more apparent in the near future.

Table 3. Cytochrome 2D6 Isoenzyme: Substrates, Inducers, and Inhibitors[2, 3, 9, 16, 19, 33, 43, 48, 58, 60, 70, 96, 114, 120–125]

Substrates			
Amitriptyline (hydroxylation)	Donepezil	Meperidine	Propafenone
Bisoprolol	Doxepin	Methadone	Propranolol
Chlorpromazine	Flecainide	Methamphetamine	Risperidone
Clomipramine	Fenfluramine	Metoprolol	Thioridazine
Clozapine	Fluphenazine	Mexiletine	Timolol
Codeine	Fluoxetine	Morphine	Tramadol
Cyclobenzaprine (hydroxylation)	Haloperidol	Nortriptyline (hydroxylation)	Trazodone
Desipramine	Hydrocodone	Oxycodone	Venlafaxine
Dexfenfluramine	Imipramine (hydroxylation)	Paroxetine	
Dextromethorphan	Maprotiline	Perphenazine	
Inhibitors		Inducers	
Amiodarone	Paroxetine		Carbamazepine
Cimetidine	Propafenone		Phenobarbital
Clomipramine	Quinidine		Phenytoin
Desipramine	Ritonavir		Rifampin
Fluoxetine	Sertraline		Ritonavir
Fluphenazine	Thioridazine		
Haloperidol			
Mibefradil			

Significant Inhibitory Interactions: Protease Inhibitors

As previously described, protease inhibitors saquinavir, ritonavir, indinavir, and nelfinavir are substrates and inhibitors of the cytochrome 3A4 system. Ritonavir is also a significant inhibitor of the 2D6 isoenzyme system (Table 3).[2, 3, 9, 16, 19, 33, 43, 48, 58, 60, 70, 114, 120–124]

These agents are likely to be given in combination with nucleosides and several other drugs (e.g., antimicrobials, antivirals) used to treat infections in patients with the acquired immunodeficiency syndrome. When comparing these agents, ritonavir appears to be a more potent inhibitor, and patients receiving it will require additional monitoring to avoid significant interactions. Indinavir appears to less potent and is a reversible inhibitor of 3A4.[125] Therefore, it may be rational to give ritonavir in the early stage of human immunodeficiency virus (HIV) disease before a patient begins receiving numerous drugs that may interact and require complex dosage alterations.

Due to the poor absorption of saquinavir, it has been studied in combination with ritonavir to increase its plasma concentrations. Ritonavir has increased saquinavir concentrations by 18-fold.[125] This concept of combination therapy with other protease inhibitors warrants further study.

Occupational exposure to HIV may now result in a 4-week course of indinavir or other protease inhibitor.[105] Little clinical information and few case reports are available regarding drug interactions with these agents, but the potential for interactions has been addressed based on pharmacokinetic in vitro data.[108] The majority of these data are with ritonavir, but prescribers should be aware that concomitant administration of drugs metabolized by the 3A4 system with other protease inhibitors may also result in interactions.

Concomitant administration of the 3A4 inhibitor ketoconazole with indinavir should include a dosage reduction of indinavir to 600 mg every 8 hours.[62] Ketoconazole increases the AUC of saquinavir by 150%, but the consistency and extent of this interaction varies widely among patients.[125] Generally, when given in combination with saquinavir, dosage adjustment is not required unless ketoconazole dosages greater than 200 mg/day are given.[61]

Fluconazole's effect on protease inhibitor concentrations is unknown, but it may not produce as large an increase in their concentrations as the addition of ketoconazole because it is not thought to be as potent of an inhibitor.[125] Similarly, concomitant administration of fluconazole 200 mg/day and ritonavir 200 mg 4 times/day resulted in insignificant changes in the half-life of ritonavir. Dosage adjustments are not necessary.[60]

Protease inhibitors increase rifabutin concentrations by inhibiting 3A4 metabolism. Ritonavir and nelfinavir increase the rifabutin AUC by 4-fold and 207%, respectively.[63–65] These increases are associated with an increased risk of side effects including uveitis, making the

Table 4. 2D6 Clinically Significant Drug Interactions[2, 3, 9, 16, 19, 33, 38, 43, 48, 58, 60, 70, 96, 114, 120–130]

Substrate	Inhibitor, Inducer, Competing Substrate	Management	Alternatives
Analgesics			
Codeine	All 2D6 inhibitors, especially quinidine	Avoid; monitor for diminished analgesic effects; higher risk in PMs and EMs taking inhibitors; reduced morphine conc by 95%.	Other analgesics
Fentanyl Hydrocodone Meperidine Methadone Oxycodone Propoxyphene	Ritonavir (inh)	Marked increase in AUC; use alternative analgesics; monitor for toxicity; especially significant for fentanyl, meperidine, propoxyphene.	
Hydrocodone Oxycodone	Rifampin (ind)	Increase dosage; well-documented reports of withdrawal.	
Meperidine Methadone	Carbamazepine (ind) Phenobarbital (ind) Phenytoin (ind) Primadone (ind)	Increase dosage; monitor for withdrawal (well documented).	Other anticonvulsants
Meperidine	Cimetidine (inh)	22% decrease in clearance with respiratory depression, sedation; not seen with morphine.	Famotidine Nizatidine Ranitidine
Tramadol	Ritonavir (inh)	May need initial dosage decrease (based on in vitro data).	
Antiarrhythmics			
Flecainide Mexiletine Propafenone	Fluoxetine (inh) Paroxetine (inh) Sertraline (inh) (less effect)	Avoid combination due to narrow therapeutic index of antiarrhythmics.	?Fluvoxamine Venlafaxine
	Amiodarone (inh)	Reduce dosage 30–50% when starting amiodarone.	
	Quinidine (inh)	EMs at greatest risk; consider dosage decrease by 50%; monitor ECG.	
Mexiletine	Ritonavir (inh)	May need initial dosage decrease (based on in vitro data).	
Mexiletine Propafenone	Rifampin (ind)	May need initial dosage increase; monitor clinical effects.	
	Phenobarbital (ind) Phenytoin (ind)	Monitor for diminished effects.	
Propafenone	Cimetidine (inh)	Reports of up to 50–75% increase in serum conc with QRS prolongation; monitor ECG.	
Antidepressants			
Amitriptyline Desipramine Doxepin Imipramine Nortriptyline Trazodone	Fluoxetine (inh) Paroxetine (inh) Sertraline (inh)	Give lower dosages in combination; monitor for SE; wait 2–4 wks after fluoxetine discontinued.	Fluvoxamine Venlafaxine
	Cimetidine (inh)	Monitor psychomotor performance; orthostatic hypotension, urinary retention other symptoms reported.	Famotidine Nizatidine Ranitidine
	Mibefradil (inh)	May require substantial dosage adjustment.	
	Carbamazepine (ind) Phenobarbital (ind) Phenytoin (ind) Primadone (ind) Chronic ETOH ingestion (ind)		Gabapentin Lamotrigine Topiramate Valproate
	Acute ETOH ingestion (inh)	Inhibits metabolism; increases serum conc.	

Table 4. 2D6 Clinically Significant Drug Interactions[2, 3, 9, 16, 19, 33, 38, 43, 48, 58, 60, 70, 96, 114, 120–130] (continued)

Substrate	Inhibitor, Inducer, Competing Substrate	Management	Alternatives
Amitriptyline Clomipramine Desipramine Imipramine Maprotiline Nortriptyline	Ritonavir (inh)	May need initial dosage decrease (based on in vitro data).	
Desipramine Imipramine Others?	Quinidine (inh)	Monitor for signs of TCA toxicity (arrhythmias, confusion, sedation); higher risk in EMs.	Other combinations desirable
Fluoxetine Paroxetine Venlafaxine	Ritonavir (inh)	May need initial dosage decrease (based on in vitro data).	
Fluoxetine	Clarithromycin (inh)	One case of delirium reported.	Azithromycin Dirithromycin
Trazodone	Paroxetine (inh) ?Other SSRIs	Reports of serotonergic syndrome.	
Antipsychotics			
Chlorpromazine Haloperidol	Ritonavir (inh)	May need initial dosage decrease (based on in vitro data).	
Perphenazine Thioridazine	Fluoxetine (inh) Paroxetine (inh) Sertraline (inh)	Increases serum conc after 7–10 days; confirmed with fluoxetine and haloperidol; monitor for side effects.	
β-Blockers			
Bisoprolol Labetalol Metoprolol	Ritonavir (inh)	May need initial dosage decrease (based on in vitro data).	Other β-blockers not metabolized (e.g., atenolol, nadolol)
Pindolol Propranolol	Rifampin (ind)	May need initial dosage increase; monitor clinical effect.	Other β-blockers
Timolol	Fluoxetine (inh) Paroxetine (inh) Sertraline (inh) (less effects) Mibefradil (inh)	Monitor for clinical effects; inhibits metabolism. Substantial dosage adjustments may be necessary.	Fluvoxamine Venlafaxine —
Propranolol	Smoking, PAH (ind)	Lower plasma conc in smokers; monitor for effect.	
	Quinidine (inh)	Higher risk in EMs.	Other β-blockers
Miscellaneous			
Cyclobenzaprine	Fluoxetine (inh)	Observe for QT prolongation.	
Dexfenfluramine Fenfluramine	Fluoxetine (inh) Fluvoxamine (inh) Paroxetine (inh) Sertraline (inh)	Avoid combination; theoretical; increased risk of serotonergic syndrome; increased risk in PMs.	

PMs = poor metabolizers; EMs = extensive metabolizers; AUC = area under the concentration-time curve; SE = side effects; PAH = polycyclic aromatic hydrocarbons; TCA = tricyclic antidepressant.

combination contraindicated.[125] Concomitant administration of indinavir with rifabutin led to a 204% increase in rifabutin AUC. Therefore, an adjustment to one-half the standard rifabutin dosage is recommended for patients receiving this combination.[62, 125] Of note, a similar interaction was observed between rifabutin and fluconazole, making monitoring for ocular side effects and uveitis essential.[125]

Benzodiazepines and opiates have demonstrated reduced clearance when given in combination with ritonavir and indinavir. If used in combination with protease inhibitors, dosages should be reduced or alternative agents should be considered.[125]

Other drugs studied concomitantly with

ritonavir and noted to have a significant (> 3-fold) increase in their AUC include benzodiazepines, calcium channel blockers, antidepressants, antiarrhythmics, corticosteroids, anticoagulants, and opiates.[125] Ritonavir increased the AUC of many 2D6-metabolized drugs by 1.5- to 3-fold (Table 4),[2, 3, 9, 16, 19, 33, 43, 48, 58, 60, 70, 114, 120–130] together with changes in some 1A2-metabolized drugs (Table 5).[2, 3, 9, 16, 18, 36, 37, 39, 40, 45, 96, 114, 117, 120, 131–139] Large dosage adjustments (50% reduction) may be required with these drugs.

Ritonavir caused a 77% increase in the AUC of clarithromycin. Prescribing information for ritonavir recommends no dosage adjustment in patients with normal renal function, a 50% reduction in clarithromycin dosage in patients with creatinine clearance (Cl_{cr}) of 30–60 ml/minute, and a 75% reduction for Cl_{cr} below 30 ml/minute. Another specific example of an AUC increase associated with ritonavir is desipramine (145% increase in AUC). Desipramine dosages should be decreased when the drug is given concurrently with ritonavir.[60]

Protease inhibitors should be prescribed cautiously in combination with drugs primarily metabolized by the 3A4 system and those metabolized by the 2D6 system (ritonavir only). Concurrent administration should be accompanied by careful clinical monitoring for side effects and dosage adjustments is some patients.

Significant Induction Interactions: Protease Inhibitors

One major interaction of concern with these drugs is rifampin's and rifabutin's induction of metabolism. Rifampin 300–600 mg/day decreased saquinavir concentrations by 80%, making saquinavir dosage adjustments necessary. Rifampin decreased the maximum concentration and AUC of ritonavir by 25% and 35%, respectively.[125] Rifabutin is a less potent inducer than rifampin, but decreased saquinavir plasma concentrations by 40%.[61, 125] The clinical significance of these decreases in protease inhibitor concentrations is unknown, but given the fact that resistance is associated with suboptimal plasma levels of these drugs, consequences could be serious.[125]

Guidelines for concomitant administration of rifampin with protease inhibitors were published recently by the Centers for Disease Control and Prevention in the *Morbidity and Mortality Weekly Report*.[59] If protease inhibitor therapy cannot be discontinued during rifampin therapy, two options are available. The first is to administer a four-drug tuberculosis regimen that includes rifampin for a minimum of 2 months or until bacteriologic response (usually 3 mo) is achieved. Rifampin can be discontinued and a modified regimen continued for 16 additional months after that time. This option cannot be attempted in isoniazid-resistant patients. Option 2 is to continue protease inhibitor therapy with indinavir 800 mg every 8 hours, or switch to indinavir if the patient is taking another protease inhibitor, and administer a four-drug, 9-month regimen that contains rifabutin 150 mg/day (one-half usual dosage) instead of rifampin. This is based on the fact that rifabutin has less inducing effect than rifampin and has comparable antituberculosis activity in vitro.[59]

Other agents that are known inducers of 3A4, including phenobarbital, phenytoin, carbamazepine, and dexamethasone, should be given cautiously with these drugs; alternatives are recommended, if possible.[125] Tobacco, another known inducer, is associated with an 18% decrease in the AUC of ritonavir.[60] Specific dosage adjustments in smokers have not been developed.

Significant Interactions: Cyclosporine

Numerous drug interactions with cyclosporine have surfaced in recent years that are associated

Table 5. Cytochrome 1A2 Isoenzyme: Substrates, Inducers, and Inhibitors[2, 3, 9, 16, 18, 36, 37, 39, 40, 45, 96, 114, 117, 120, 131–139]

Substrates	Inhibitors
Amitriptyline (demethylation)	Cimetidine
Caffeine	Ciprofloxacin
Clomipramine (demethylation)	Clarithromycin
Clozapine	Enoxacin
Cyclobenzaprine (demethylation)	Erythromycin
Desipramine (demethylation)	Fluvoxamine (potent)
Diazepam	Grapefruit juice
Haloperidol	Isoniazid
Imipramine (demethylation)	Ketoconazole
Tacrine	Levofloxacin
Theophylline	Norfloxacin
R-warfarin	Omeprazole?
Zileuton	Paroxetine
Inducers	
Phenobarbital	
Phenytoin	
Rifampin	
Ritonavir	
Smoking/PAH	

PAH = polycyclic aromatic hydrocarbons.

with its metabolism and presystemic metabolism by the 3A4 enzyme in the liver and intestine, respectively. It is postulated that gastrointestinal tract metabolism may in part explain its erratic absorption.[107–109] In fact, 3A4 inhibition has been given intentionally to improve cyclosporine's bioavailability and decrease its dosage requirements. Ketoconazole 200–400 mg/day can decrease dosage requirements by 60–80%. Serum concentrations begin to increase within 2 days, but 2–4 weeks may be required for stabilization.[110, 111]

Diltiazem in variable dosages decreased cyclosporine dosage requirements by as much as 30%.[112] In some studies, grapefruit juice increased the drug's AUC by 19–60% when given within 90 minutes before or after cyclosporine.[52, 56] However, other authors showed no change in cyclosporine AUC or trough concentrations with concomitant administration of grapefruit juice 1.5 L/day.[55] Therefore, the effect with grapefruit juice is highly variable, and the clinical significance is unknown.

A study in pediatric renal transplant recipients showed that lower dosages of cyclosporine are required in patients also receiving norfloxacin. This has not been seen with ciprofloxacin.[113]

Other drugs that alter cyclosporine concentrations secondary to cytochrome 3A4 inhibition include verapamil, nicardipine,[107–109] fluconazole, itraconazole, ketoconazole, erythromycin, clarithromycin,[33, 108, 109] tacrolimus,[108, 109] and mibefradil.[38] Cyclosporine concentrations are decreased secondary to enzyme induction with rifampin,[16, 33, 108, 109, 114] phenytoin, carbamazepine, and phenobarbital.[108, 109, 120] Cyclosporine trough levels, signs of toxicity, and adequate immunosuppressive response should be monitored when these drugs are begun or discontinued in combination with cyclosporine.

Significant Induction Interactions: Estrogens and Corticosteroids

Reports of breakthrough bleeding and unintended pregnancies due to drug interactions are increasing, perhaps due to the fact that estrogen and progestin concentrations of oral contraceptives are decreased. Clinically significant drug interactions with oral contraceptives secondary to 3A4 enzyme induction include carbamazepine, ethosuximide, phenobarbital, phenytoin, primidone, and rifampin,[114, 115, 117, 120] They have not been reported with gabapentin, lamotrigine, topiramate, and valproate. With these interactions, a higher-dose oral contraceptive (50 µg ethinyl estradiol), medroxyprogesterone, or nonhormonal alternative method of contraception is desirable.

Similarly, corticosteroid clearance is increased with the same concomitant agents. Patients receiving corticosteroids for chronic diseases should be monitored for exacerbation of symptoms in these situations.[120]

Other potential interactions are with benzodiazepines, which also may compete with or induce microsomal hepatic enzymes. They may reduce the effectiveness of oral contraceptives, whereas oral contraceptives can enhance the effect of benzodiazepines by competing with their clearance. During short courses of benzodiazepines, an alternative method of contraception is desirable. Oral contraceptives themselves reduce prednisolone clearance up to 50%, increase serum phenytoin concentrations, decrease metoprolol clearance, and reduce theophylline clearance up to 33%.[117] How clinically significant these interactions are remains to be determined.

Significant Inhibitory Interactions: Corticosteroids

Little information is available regarding inhibition of estrogen or corticosteroid metabolism. Ketoconazole increases the AUC of both prednisolone and methylprednisolone in humans through inhibition of metabolism. Further studies are required to clarify the clinical significance of this alteration.[33]

2D6 Isoenzyme

The 2D6 isoenzyme is the most intensely studied CYPP450 enzyme since a genetic polymorphism in its drug metabolism was identified over 15 years ago. Administration of dextromethorphan followed by measurement of *O*-demethylated metabolite excretion in urine is an accurate and noninvasive way of phenotyping individuals as either EMs or PMs for 2D6 activity. The PMs lack this enzyme as a result of an autosomal recessively transmitted defect in its expression. Of note, approximately 5–10% of whites are PMs compared with 1–3% of African-Americans and Asians.[2, 3]

For drugs that are highly dependent on clearance to an inactive metabolite by 2D6, PMs may have a larger response and be at greater risk of toxicity than EMs. For example, there is an association between PMs and tricyclic antidepressant-induced cardiotoxicity and with neuroleptic-induced side effects.[19] Furthermore,

inhibition can reduce the metabolic rate in an EM to a value comparable with that of a PM.[2, 5, 9, 40] When drugs are converted to an active metabolite by 2D6 (e.g., conversion of codeine to morphine), the drug may be ineffective in PMs. Induction cannot convert PMs to EMs, because only inactive or relatively inactive forms of the enzyme can be induced. To date, specific inducers of 2D6 have yet to be clearly identified,[21, 9] but significant interactions between 2D6-metabolized drugs with the well-known inducers rifampin[114] and anticonvulsants[120] have been described for years.

Large numbers of drugs affecting the cardiovascular and central nervous systems have been identified as substrates for 2D6 (Table 3).[2, 9, 39, 40, 43, 70, 96, 121, 122, 124]

Significant Inhibitory Interactions: Antidepressants and Antipsychotics

In vivo, the selective serotonin reuptake inhibitor (SSRI) antidepressants fluoxetine and paroxetine are equipotent inhibitors of 2D6. Sertraline has less pronounced inhibition, and fluvoxamine is almost devoid of inhibitory effects.[9, 122, 132] Coadministration with tricyclic antidepressants (TCAs) has been a focus of interest since these drugs are coadministered in some cases for resistant patients. Administration of desipramine with fluoxetine 20 mg/day and paroxetine 20 mg/day produced up to 4-and 3-fold increases, respectively, in peak serum concentrations. Similar results were shown with nortriptyline and imipramine.[9] This inhibition is reversed within 1 week of discontinuing paroxetine, 1–2 weeks with sertraline, and up to 5 weeks with fluoxetine because of the prolonged half-lives of the parent compound and its metabolite.[9, 19]

On average, the percentage increase in TCA plasma concentrations over baseline has ranged from 58–150% with sertraline 50 mg/day and 110–375% with fluoxetine 20 mg/day.[19] Clinical sequelae resulting from the coadministration of SSRIs and TCAs have been reported only rarely, but full dosages of both agents could clearly lead to plasma concentrations in the toxic range. A summary of 25 cases involving combinations of fluoxetine and various TCAs showed that the magnitude of increased TCA concentrations is variable, does not correlate with the occurrence of adverse effects, and is not predictable.[124] Lower dosages with these combinations along with careful monitoring for side effects seem warranted (Table 4).[2, 3, 9, 16, 19, 33, 38, 43, 48, 58, 60, 70, 114, 120–130] Paroxetine was reported to interact with trazodone, with serotonergic syndrome occurring with 24 hours of administration of paroxetine 20 mg.[19]

We encountered a potential drug interaction in our institution that may have been mediated partly by fluoxetine inhibition of the 2D6 system. A 59-year-old woman was admitted for Achilles tendon repair. Her baseline QT interval on admission was prolonged (497 msec). Her drugs before admission included cyclobenzaprine, fluoxetine, diclofenac, amlodipine, and triamterene-hydrochlorothiazide. She had no known history of cardiac disorders except for hypertension. During outpatient surgery she had sudden onset of torsades de pointes that deteriorated into ventricular fibrillation. Preoperatively, she received droperidol, which is known to potentiate QT prolongation and should be given cautiously to patients with baseline QT prolongation.[126] The woman converted to normal sinus rhythm with magnesium sulfate and defibrillation. All drugs were discontinued, and her QT interval returned to below baseline levels by postoperative day 1. The question remained about the etiology of her baseline QT prolongation; we postulated inhibition of cyclobenzaprine metabolism by fluoxetine.

Cyclobenzaprine is hepatically metabolized, has a structure similar to TCAs (2D6, 3A4, 1A2 metabolized),[96] and caused conduction abnormalities in cases of overdose. In addition, its average half-life is 24 hours, which corresponds to the gradual decrease in the patient's QT interval.[127] Fluoxetine is a known inhibitor of 2D6, 3A4, and 2C, and reportedly increases serum concentrations of TCAs due to these effects.[9, 19, 122–124] Although not documented, the potential exists for inhibition of cyclobenzaprine metabolism by fluoxetine.

Interactions secondary to enzyme inhibition by SSRIs and that are documented by pharmacokinetic studies and case reports occurred with flecainide, propafenone, haloperidol, and other antipsychotics. Due to the narrow therapeutic index and high-risk patients who receive type Ic antiarrhythmics, SSRIs should be avoided in patients taking these drugs.[19] Vigilant pharmacodynamic monitoring should accompany therapy with SSRIs in patients treated with other drugs metabolized by the 2D6 enzyme.

Although clarithromycin has not been identified as a 2D6 substrate or inhibitor, a report of delirium in a 53-year-old man who was receiving long-term fluoxetine 80 mg/day and

clarithromycin indicates this potential. The patient's delirium quickly cleared after he stopped the drugs, and did not recur with erythromycin alone or when he restarted fluoxetine 80 mg/day. The authors concluded that the delirium was consistent with fluoxetine intoxication, which could have resulted from inhibition of metabolism by clarithromycin.[128]

Other potential interactions with TCAs include mibefradil and quinidine. Quinidine is the most potent 2D6 inhibitor identified to date.[39] It inhibited TCA metabolism and resulted in 85% reduction in desipramine clearance and a 35% decrease in imipramine clearance.[129] Mibefradil is a CYP2D6 inhibitor and can increase plasma levels of TCAs, necessitating substantial dosage reductions.[38] Patients receiving these combinations should be monitored for signs of TCA toxicity.

Significant Induction Interactions: Narcotic Analgesics

Several opioids including meperidine, methadone, and morphine are metabolized by the 2D6 enzyme. Several well-documented interactions result from enzyme induction and loss of opioid activity.[70] Rifampin 600–900 mg/day precipitated withdrawal symptoms when given to 21 patients with tuberculosis receiving methadone maintenance.[130] Similar effects were reported with rifampin 450 mg/day.[16, 70] Consideration should be given to opioid dosage increases when rifampin is begun, and patients should be monitored closely for symptoms of withdrawal. Similar enzyme-inducing effects and signs of narcotic withdrawal with up to 50% reduction in methadone concentrations were documented with methadone and phenytoin, phenobarbital, and carbamazepine. Pharmacokinetic studies also indicated that reductions in the clearance of meperidine could be expected with these enzyme-inducing agents.[70, 120]

Pharmacodynamic monitoring would appear to be the most appropriate management strategy when narcotics are given concurrently with enzyme-inducing agents.[2, 3, 9, 16, 19, 33, 43, 48, 58, 60, 70, 114, 120–130]

Significant Inhibitory Interactions: Narcotic Analgesics

Coadministration of cimetidine 1200 mg/day with meperidine decreased meperidine clearance by up to 22%.[70]

Special care should be taken when administering codeine with 2D6 inhibitors or when no clinical effect is achievable. Codeine is a prodrug and 10% of the dose is *O*-demethylated to the active metabolite, morphine. This demethylation is impaired in PMs and reduced in EMs during treatment with inhibitor drugs.[3] The combination should probably be avoided since diminution of codeine's effect is is highly probable.

1A2 Isoenzyme

The 1A2 isoenzyme is of clinical interest because of the large number of drug interactions associated with theophylline dealkylation and because of its inducibility by PAH in cigarette smoke and charcoal-broiled foods. No genetic polymorphism has been defined but possibly exists because of observations of a trimodal pattern of caffeine metabolism. The 1A2 enzyme is also responsible for metabolism of the R-isomer of warfarin as well as with several benzodiazepines. As noted in Table 5,[2, 3, 9, 16, 18, 36, 37, 39, 40, 45, 96, 114, 117, 120, 131–139] drugs known to be inhibitors of 1A2 include fluvoxamine (very potent),[2, 19, 39, 132] cimetidine,[13, 131] macrolides,[36, 37, 131] and several of the quinolones.[131,134–136]

Significant Inhibitory Interactions: Theophylline

Cimetidine is an enzyme inhibitor and causes a pharmacokinetic interaction with theophylline (theophylline clearance is decreased approximately 30%). Pharmacodynamic data are lacking, however. An initial dosage reduction should be considered in patients with a baseline theophylline level above 12 µg/ml due to theophylline's narrow therapeutic index.[13, 131]

Fluvoxamine is a potent inhibitor of 1A2, unlike the other SSRIs. It increased theophylline concentrations 2- to 3-fold, along with significant increases in haloperidol and clozapine concentrations.[19, 132] The majority of patients had increased plasma theophylline concentrations accompanied by clinical symptoms.[132]

Interactions with macrolides are fairly well documented. In most studies, erythromycin and clarithromycin decreased theophylline clearance 20–25% after 7 days of concomitant administration. Most clinicians recommend theophylline dosage reduction and careful monitoring if the baseline theophylline level is above 12 µg/ml. Other macrolides such as azithromycin and dirithromycin are routinely suggested as alternatives.[36, 37, 131] In one patient, however, addition of azithromycin to a maintenance theophylline regimen resulted in an increase in serum concentration from the usual 12.7–15.5

μg/ml to 20 μg/ml, and discontinuation of azithromycin resulted in a 80% decrease in the concentration. This was confirmed with two rechallenges.[132] Thus, clinicians should be aware of the potential for an interaction with azithromycin.

Several quinolones commonly decrease theophylline clearance. Enoxacin has the greatest potential, with a 50–65% reduction in clearance, followed by ciprofloxacin with a 25–30% decrease and norfloxacin with a 10–15% decrease.[75, 131, 134–136] These interactions resulted in symptoms of theophylline toxicity including seizures. Up to 50% dosage reduction is recommended for patients with a baseline theophylline level above 12 μg/ml when beginning these combinations.[129, 130, 132–134] Clinically significant interactions with ofloxacin, levofloxacin, lomefloxacin, and sparfloxacin are unusual, making them alternative quinolones.

Isoniazid also decreases theophylline clearance after at least 6 days of concomitant administration. This appears to be most pronounced in slow acetylators, with up to a 2-fold increase in theophylline concentrations.[16, 131]

Oral contraceptives decrease theophylline clearance by 30%, necessitating more careful monitoring when starting or discontinuing concomitant therapy with theophylline. The proposed mechanism is inhibition of metabolism.[117] Grapefruit juice has no effect on theophylline metabolism, although it may have some 1A2 inhibitory effects.[131]

Significant Induction Interactions: Theophylline

The PAH in cigarette smoke induce 1A2 enzymes responsible for theophylline metabolism. It was estimated that smokers may require up to twice the dosage relative to that of nonsmokers, and a dosage reduction by one-fourth to one-third during abstinence. Some reports indicate that enzyme induction is present for up to several months after smoking cessation.[18, 131]

Other well-established induction interactions with theophylline including rifampin and the anticonvulsant drugs carbamazepine, phenobarbital, and phenytoin[114, 120, 131] are summarized in Table 6.[2, 3, 9, 16, 18, 36, 37, 39, 40, 45, 114, 117, 120, 131–139]

Significant Inhibitory Interactions: R-Warfarin

As discussed, the R-isomer of warfarin is the less pharmacologically active form, but significant drug interactions have resulted from inhibition of its metabolism. The R-isomer is partially metabolized by the 3A4 system, and several of these drug interactions have been discussed.

A series of case reports described the interaction between warfarin and quinolones that can occur as early as day 2 or as late as day 16 after beginning quinolone therapy. Hemorrhagic complications attributed to this interaction have been reported as well. Ciprofloxacin, norfloxacin, ofloxacin (least likely), nalidixic acid, and enoxacin were implicated in these reports. According to pharmacokinetic studies, the interaction is probably secondary to inhibition of the R-stereoisomer of warfarin, which is partly metabolized by the 1A2 isoenzyme.[33, 135, 136] However, several prospective, placebo-controlled trials showed no clinically significant effect.[138, 139] Since warfarin is metabolized by enzymes from three different families, most individuals may be able to shunt its metabolism to a noninhibited pathway. Although this interaction may be rare and unpredictable, careful monitoring of the INR is warranted during concomitant therapy (Table 6).[2, 3, 9, 16, 18, 36, 37, 39, 40, 45, 114, 117, 120, 131–139] Alternative quinolones with little to no inhibitory properties include levofloxacin, lomefloxacin, and sparfloxacin.

The manufacturer of fluvoxamine has received 11 reports of interactions with warfarin. The drug increases the measured warfarin concentrations by 65%; all patients in case reports had elevated prothrombin times and some had bleeding complications.[132] The safety of combining warfarin with other SSRIs was studied in patients previously maintained with warfarin. Fluoxetine appears to have no effect, and both paroxetine and sertraline caused an increase in prothrombin time, with reports of minor bleeding with paroxetine.[19, 43] Close INR monitoring is warranted during therapy with any SSRI.

Warfarin metabolism is known to be inhibited by cimetidine, but data on pharmacodynamic and clinical effects are lacking. It seems prudent to monitor the INR carefully during combination therapy or to consider an alternative histamine$_2$ (H_2) antagonist.[13] The enzyme-inhibitory effects of cimetidine are attributed to its imidazole ring.

Significant Inhibitory Interactions: Antidepressants, Antipsychotics, and Benzodiazepines

Fluvoxamine increases plasma concentrations with clinical symptoms of toxicity (e.g., confusion, tremor, extrapyramidal syndrome) in patients receiving amitriptyline (2-fold increase), clomipramine (8-fold), clozapine (3.2- to 11.8-

Table 6. 1A2 Clinically Significant Drug Interactions[2, 3, 9, 16, 18, 36, 37, 39, 40, 45, 114, 117, 120, 131–139]

Substrate	Inhibitor, Inducer, Competing Substrate	Management	Alternatives
Theophylline	Rifampin (ind)	Can occur within 2–4 days; may need initial dosage increase; monitor serum conc.	
	Erythromycin (inh) Clarithromycin (inh) Troleandomycin (inh)	Usually not seen for 7 days but reported as early as 2 days; more careful monitoring if baseline level ≥ 12 µg/ml.	Azithromycin Dirithromycin
	Ritonavir (ind)	Decrease in theophylline AUC by 43%; increased theophylline dosage may be required; monitor serum conc.	
	Enoxacin (inh) Ciprofloxacin (inh) Norfloxacin (inh)	Seen in 2–6 days; consider decreasing dosage 30–50% if baseline level ≥ 12 µg/ml; check level 2 days into therapy.	Levofloxacin Lomefloxacin Ofloxacin Sparfloxacin
	Fluvoxamine (inh)	Confirmed by reports; monitor SC&E.	Fluoxetine Paroxetine Sertraline Venlafaxine
	Cimetidine (inh)	Can occur within 24 hrs; reduce initial dosage 40% if baseline level ≥ 12 µg/ml.	Famotidine Nizatidine Ranitidine
	Isoniazid (inh)	Up to 2-fold increase in serum conc; more pronounced in slow acetylators; monitor serum conc.	
	Oral contraceptives (inh)	Decreased clearance 30%; more significant if > 35 µg estrogen.	
	Zileuton (inh)	Reported to reduce clearance; monitor more carefully.	
	Smoking, PAH (ind)	Increase initial dosage by 50%; monitor serum conc; effects may persist for 3 mo after smoking cessation.	
	Carbamazepine (ind) Phenobarbital (ind) Phenytoin (ind)	Monitor serum conc more carefully; can see within 5 days with phenytoin.	Gabapentin Lamotrigine Topiramate Valproate
Anticoagulant R-warfarin	Ciprofloxacin (inh) Enoxacin (inh) Nalidixic acid (inh) Norfloxacin (inh)	Occurs in 2–16 days; unpredictable but can be clinically significant; monitor INR more carefully.	Levofloxacin Lomefloxacin Ofloxacin Sparfloxacin
	Cimetidine (inh)	Dose dependent with at least 400–800 mg/day cimetidine; monitor INR more carefully.	Famotidine Nizatidine Ranitidine
	Fluvoxamine (inh) (most potent) Fluoxetine (inh) Paroxetine (inh) Sertraline (inh)	Many case reports of increased INR with bleeding.	
	Zileuton (inh)	Increased INRs reported; monitor more carefully.	
Antidepressants and antipsychotics Amitriptyline Clomipramine Desipramine Imipramine	Fluvoxamine (inh)	Cases of increased serum conc with clinical symptoms of confusion, tremor.	

Table 6. 1A2 Clinically Significant Drug Interactions[2, 3, 9, 16, 18, 36, 37, 39, 40, 45, 114, 117, 120, 131–139] (continued)

Substrate	Inhibitor, Inducer, Competing Substrate	Management	Alternatives
Chlordiazepoxide Diazepam Others?	Smoking, PAH (ind)	Less drowsiness in smokers; may require higher dosages.	
Clozapine Haloperidol	Fluvoxamine (inh)	Avoid combination; resulted in markedly increased serum conc with symptoms of EPS.	Fluoxetine Paroxetine Sertraline
Miscellaneous			
Tacrine	Smoking, PAH (ind)	Mean plasma conc 1/3 less in smokers; may require higher dosages.	
	Cimetidine (inh)	Decreases clearance 30%; monitor for anticholinergic side effects.	Famotidine Nizatidine Ranitidine
	Enoxacin (inh) Ciprofloxacin (inh) Norfloxacin (inh)	Theoretical.	Lomefloxacin Ofloxacin Sparfloxacin

AUC = area under the concentration-time curve; SC&E = serum concentration and effects; INR = international normalized ratio; PAH = polycyclic aromatic hydrocarbons; EPS = extrapyramidal syndrome.

fold), desipramine (1-fold), and imipramine (1.3- to 5.7-fold).[132] Patients receiving these combinations should be monitored for side effects and toxicity.

Significant Induction Interactions: Benzodiazepines

Similar to theophylline, the inducing effects of smoking are associated with less drowsiness in patients taking chlordiazepoxide and diazepam. This was studied in a comprehensive in-hospital drug surveillance program comparing 2274 nonsmokers, light smokers, and heavy smokers receiving these benzodiazepines. Smokers may require larger dosages of benzodiazepines to achieve a sedative or anxiolytic effect.[18]

2C Isoenzyme

The 2C subfamily consists of isoenzymes 2C9, 2C10, 2C19, and others. Cytochrome 2C19 exhibits genetic polymorphism, with 20% of Asians and African-Americans and 3–5% of Caucasians reported as PMs.[2, 19] Drugs metabolized by the 2C subfamily include phenytoin (2C9), S-warfarin (2C9), and omeprazole. Diazepam, clomipramine, amitriptyline, and imipramine are demethylated by 2C enzymes. Known inhibitors of 2C enzymes include amiodarone (2C9) and omeprazole (2C19). Fluvoxamine, fluoxetine, and sertraline may inhibit these enzymes on the basis of increases in plasma concentrations of drugs believed to be metabolized by this subfamily.[19, 130] In addition, as noted in Table 7,[2, 13, 65–68, 120, 132, 140–143] chloramphenicol,[33, 120] cimetidine,[13, 120] and isoniazid[16, 120] are probable inhibitors on the basis of significant interactions with phenytoin.

Significant Inhibitory Interactions: Phenytoin

Case reports of 26 patients with steady-state phenytoin concentrations described a 67–309% increase in serum concentrations with the addition of the inhibitor fluoxetine within 5–13 days. Symptoms of toxicity occurred.[19] Fluvoxamine, although a better recognized 3A4 and 1A2

Table 7. Cytochrome 2C Isoenzyme: Substrates, Inducers, and Inhibitors[2, 13, 16, 33, 65–68, 120, 132, 140–143]

Substrates	Inhibitors
Amitriptyline	Amiodarone (2C9)
Clomipramine	Chloramphenicol (2C9)
Diazepam (demethylation 2C9)	Cimetidine (2C9)
Imipramine	Fluconazole
Losartan (2C9)	Fluoxetine
Omeprazole	Fluvastatin
Phenytoin (2C9)	Fluvoxamine (2C9, potent)
S-warfarin (2C9)	Isoniazid
Tolbutamide	Ketoconazole (weak)
Topiramate (2C19)	Omeprazole (2C9, 2C19)
	Sertraline
	Topiramate (2C19)
Inducers	Zafirlukast (2C9)
Carbamazepine	
Phenobarbital	
Phenytoin	
Rifampin	

Table 8. 2C Clinically Significant Drug Interactions[2, 11, 13, 15, 16, 19, 33, 65–68, 120, 132, 140–143]

Substrate	Inhibitor, Inducer, Competing Substrate	Management	Alternatives
Anticonvulsants			
Phenytoin	Rifampin (ind)	Monitor serum conc more carefully when starting and stopping therapy.	
	Isoniazid (inh)	Monitor serum conc more carefully; more pronounced in slow acetylators; monitor for ataxia, nystagmus, drowsiness.	
	Cimetidine (inh) ?Ranitidine (inh)	Dose dependent; mild phenytoin intoxication in some cases; monitor SC&E; adjust dosages as necessary.	Famotidine Nizatidine
	Omeprazole (inh)	Up to 30% increase in half-life; monitor serum conc carefully or give alternative.	Lansoprazole
	Fluconazole (inh)	Predictable increase in serum conc after 14 days; monitor levels carefully.	
	Chloramphenicol (inh)	Up to 2-fold increase in serum conc.	Other antimicrobials
	Amiodarone (inh)	2–3-fold increase in serum conc within 3–4 weeks; reduce dosage based on serum conc.	
	Topiramate (inh)	25% increase in serum conc in some pts; monitor serum conc.	
	Fluoxetine (inh) Fluvoxamine (inh)	Reports of serious toxicity with nausea, vomiting, vertigo; avoid if possible.	
Anticoagulants			
S-Warfarin (more pharmacologically active isomer)	Rifampin (ind)	Seen within 2–4 days; monitor INR daily when starting and stopping therapy	
	Carbamazepine (ind) Phenobarbital (ind) Phenytoin (ind)		Gabapentin Lamotrigine Topiramate Valproate
	Chloramphenicol (inh)	Monitor INR more carefully when starting and stopping therapy.	Other antimicrobials
	Metronidazole (inh)	Bleeding episodes reported; monitor INR more carefully.	Other antimicrobials
	Amiodarone (inh)	Delayed interaction 1 wk–2 mo; decrease dosage 25% when starting therapy.	
	Zafirlukast (inh)	Mean PT increase 35%; monitor PT more carefully.	
Benzodiazepines			
Diazepam	Fluoxetine (inh) Fluvoxamine (inh)		
Diazepam	Omeprazole (inh)	In vivo increases in serum conc; monitor for SE.	Lansoprazole
Miscellaneous			
Topiramate	Carbamazepine (ind) Phenobarbital (ind) Phenytoin (ind) Valproate (ind)	Reductions in serum conc observed.	

SC&E = serum concentration and effects; INR = international normalized ratio; PT = prothrombin time; SE = side effects.

inhibitor, may also have some 2C enzyme-inhibitory effects. The manufacturer has received reports of drug interactions with phenytoin that included nausea, vomiting, and vertigo.[132] Due to phenytoin's narrow therapeutic window and nonlinear pharmacokinetics, these combinations should be avoided or phenytoin dosages reduced (Table 8).[2, 11, 13, 15, 16, 19, 33, 65–68, 120, 132, 140–143]

Cases of mild phenytoin intoxication were reported when taken concomitantly with cimetidine. Other H_2 antagonists are without these effects and would be more desirable choices.[13, 120] A study in eight healthy volunteers showed impaired elimination of phenytoin after omeprazole 40 mg/day for 8 days. Phenytoin's elimination half-life was increased by an average of 27%.[65] Patients taking these combinations should be monitored closely, or lansoprazole may be given as an alternative in patients receiving phenytoin.[66, 143]

An increase in phenytoin concentration seems to be predictable when fluconazole is added. Nystagmus and ataxia occurred in two patients with excessive phenytoin concentrations after initiation of fluconazole 200–400 mg/day.[140] Studies in healthy volunteers showed up to 75% increase in AUC and 128% increase in trough phenytoin concentrations after 14 days of fluconazole.[33, 120]

Isoniazid is another inhibitor that increases phenytoin concentrations. This interaction seems to be most pronounced in slow acetylators.[16] A new antiepileptic agent, topiramate, also increases phenytoin serum concentrations up to 25% in some patients. Patients should be monitored carefully when receiving this combination antiepileptic regimen.[141]

Significant Induction Interactions: Phenytoin

Administration of rifampin with phenytoin and other anticonvulsants can cause therapeutic failure due to enzyme induction. Serum concentrations should be monitored regularly when beginning or discontinuing rifampin with these regimens.[16, 114]

Significant Inhibitory Interactions: S-Warfarin

The S-isomer of warfarin is metabolized by the 2C9 isoenzyme. A significant interaction that appears to be 2C9 mediated is with amiodarone and warfarin. Amiodarone decreases the total body clearance of both R- and S-warfarin. Any alterations in clearance of the R-isomer could be due to amiodarone inhibition of 3A4. The interaction can be seen from 1 week to 2 months after starting amiodarone and may persist 1–3 weeks after discontinuation. Most clinicians recommend reducing the warfarin dosage by 25% when beginning amiodarone therapy.[11, 142]

Significant Induction Interactions: S-Warfarin

Patients previously stabilized with warfarin can suffer failed anticoagulation with the addition of rifampin, or overanticoagulation when rifampin is discontinued.[16, 114] Similar problems exist with coadministration of carbamazepine, phenobarbital, and phenytoin.[120]

Significant Inhibitory Interactions: Benzodiazepines

In vivo, omeprazole inhibits the metabolism of diazepam and increases the elimination half-life of diazepam by an average of 130%. It is not entirely clear, but the interaction may be 2C9 mediated. Patients receiving this combination over the long term should be monitored for side effects, or alternative agents should be considered.[65]

Summary

Our knowledge of and ability to predict drug interactions have improved with growing understanding of substrates, inhibitors, and inducers of CYP-450 isoenzymes. This review underscores the need for definitive in vivo drug interaction studies and continued patient reporting by clinicians, since in vitro data are not always consistent with in vivo experience and since many variables (age, hepatic function, multiple metabolic pathways) influence patient outcomes. The information in this review should help health care providers in making decisions to manage CYP-450 drug interactions. Clinicians should be cognizant of potential interactions and become familiar with the substrates, inhibitors, and inducers of the common enzymatic pathways responsible for drug metabolism.

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References

1. Brouwer KLR, Dukes GE, Powell JR. Influence of liver function on drug disposition. In: Evans WE, Schentag JJ,

Jusko WJ, eds. Applied pharmacokinetics. Spokane, WA: Applied Therapeutics, 1993:6-1–59.
2. Slaughter RL, Edwards DJ. Recent advances: the cytochrome P450 enzymes. Ann Pharmacother 1995;29:619–24.
3. Brosen K. Recent developments in hepatic drug oxidation: implications for clinical pharmacokinetics. Clin Pharmacokinet 1990;18:220–39.
4. Guengerich FP. Catalytic selectivity of human cytochrome P450 enzymes: relevance to drug metabolism and toxicity. Toxicol Lett 1994;70:133–8.
5. Nebert DW, Adesnich M, Coon MJ, et al. The P450 gene superfamily: recommended nomenclature. DNA 1987;6:1–11.
6. Coutts RT. Polymorphism in the metabolism of drugs, including antidepressant drugs: comments on phenotyping. J Psychiatry Neurosci 1994;19:30–44.
7. Kunze KL, Wienkers LC, Thummel KE, Trater WF. Warfarin-fluconazole. I. Inhibition of the human cytochrome P450-dependent metabolism of warfarin by fluconazole: in vitro studies. Drug Metab Dispos 1996;24:414–21.
8. Black DJ, Kunze KL, Wienkers LC, et al. Warfarin-fluconazole. II. A metabolically based drug interaction: in vitro studies. Drug Metab Dispos 1996;24:422–8.
9. Ereshefsky L, Riesenman C, Francis Lam YW. Antidepressant drug interactions and the cytochrome P450 system: the role of cytochrome P450 2D6. Clin Pharmacokinet 1995;29(suppl 1):10–19.
10. Cooke CE, Sklar GE, Nappi JM. Possible pharmacokinetic interaction with quinidine: ciprofloxacin or metronidazole? Ann Pharmacother 1996;30:364–6.
11. Heimark LD, Wienkers L, Dunze K, et al. The mechanism of the interaction between amiodarone and warfarin in humans. Clin Pharmacol Ther 1992;51:398–407.
12. Soto J, Alsar MJ, Sacristan JA. Assessment of the time course of drugs with inhibitory effects of hepatic metabolic activity using successive salivary caffeine tests. Pharmacotherapy 1995;15:781–4.
13. Shinn AF. Clinical relevance of cimetidine drug interactions. Drug Saf 1992;7:245–67.
14. Pirttiaho HI, Sotaniemi EA, Pelkonen RO, et al. Hepatic blood flow and drug metabolism in patients on enzyme-inducing anticonvulsants. Eur J Clin Pharmacol 1982;22:441–5.
15. Harder S, Thurmann P. Clinically important drug interactions with anticoagulants. an update. Clin Pharmacokinet 1996;30:416–44.
16. Grange JM, Winstanley PA, Davies PDO. Clinically significant drug interactions with antituberculosis agents. Drug Saf 1994;11:242–51.
17. Cupp MJ, Tracy TS. Role of the cytochrome P450 3A subfamily in drug interactions. US Pharmacist 1997:HS 9–21.
18. Schein JR. Cigarette smoking and clinically significant drug interactions. Ann Pharmacother 1995;29:1139–48.
19. Nemeroff CB, DeVane L, Pollock BG. Newer antidepressants and the cytochrome P450 system. Am J Psychiatry 1996;153:311–20.
20. Honig PK, Woosley RL, Zamani K, Conner DP, Cantilena LR. Changes in the pharmacokinetics and electrocardiographic pharmacodynamics of terfenadine with concomitant administration of erythromycin. Clin Pharmacol Ther 1992;52:213–18.
21. Mathews DR, McNutt B, Okerholam R, Flicker M, McBride G. Torsades de pointes occurring in association with terfenadine use. JAMA 1991;266:2375–6.
22. Honig PK, Wortham DC, Zamani K, Conner DP, Mullin JC, Cantilena LR. Terfenadine-ketoconazole interaction: pharmacokinetic and electrocardiographic consequences. JAMA 1993;269:1513–18.
23. Monahan BP, Ferguson CL, Killeavy ES, Lloyd BK, Troy J, Cantilena LR. Torsades de pointes occurring in association with terfenadine use. JAMA 1990;264:2788–90.
24. Pratt CM, Ruberg S, Morganroth J, et al. Dose-response relation between terfenadine (Seldane) and the QT interval on the scalar electrocardiogram: distinguishing a drug effect from spontaneous variability. Am Heart J 1996;131:472–80.
25. Woosley RL. Cardiac actions of antihistamines. Annu Rev Pharmacol Toxicol 1996;36:233–52.
26. Francois I, De Nutte N. Nonulcer dyspepsia: effect of the gastrointestinal prokinetic drug cisapride. Curr Ther Res 1987;41:891–8.
27. Wysowski DK, Bacsanyi J. Cisapride and fatal arrhythmia. N Engl J Med 1996;335:290–1.
28. Inman W, Kubota K. Tachycardia during cisapride treatment. BMJ 1992;305:1019.
29. Bran S, Murray W, Hirsch IB, Plamer JP. Long QT syndrome during high-dose cisapride. Arch Intern Med 1995;155:765–8.
30. Olsson S, Edwards IR. Tachycardia during cisapride treatment. BMJ 1992;305:748–9.
31. Abramowicz M, ed. Fexofenadine. Med Lett 1996;38:95–6.
32. Kivisto KT, Neuvonen PJ, Klotz U. Inhibition of terfenadine metabolism: pharmacokinetic and pharmacodynamic consequences. Clin Pharmacokinet 1994;27:1–5.
33. Gillum JG, Isreal DS, Polk RE. Pharmacokinetic drug interactions with antimicrobial agents. Clin Pharmacokinet 1993;25:450–82.
34. Cantilena LR, Sorrels S, Wiley T, Wortham D. Fluconazole alters terfenadine pharmacokinetics and electrocardiographic pharmacodynamics [abstr]. Clin Pharmacol Ther 1995; 57:185.
35. Harris S, Hilligoss DM, Colangelo PM, Eller M, Okerholm R. Azithromycin and terfenadine: lack of drug interaction. Clin Pharmacol Ther 1995;58:310–15.
36. Amsden GW. Macrolides versus azalides: a drug interaction update. Ann Pharmacother 1995;29:906–17.
37. van-Rosenteil NA, Adam D. Macrolide antibacterials. Drug interactions of clinical significance. Drug Saf 1995;13:105–22.
38. Abramowicz M, ed. Mibefradil—a new calcium-channel blocker. Med Lett 1997;39:103–5.
39. DeVane L. Pharmacokinetics of the newer antidepressants: clinical relevance. Am J Med 1994;97(suppl 6A):13S–23.
40. Shen WW. Cytochrome P450 monooxygenases and interactions of psychotropic drugs: a five-year update. Int J Psychiatry Med 1995;25:277–90.
41. Swims MP. Potential terfenadine-fluoxetine interaction. Ann Pharmacother 1993;27:1404–5.
42. Marchiando RJ, Cook MD. Probable terfenadine-fluoxetine-associated cardiac toxicity. Ann Pharmacother 1995;29: 937–8.
43. Finley PR. Selective serotonin reuptake inhibitors: pharmacologic profiles and potential therapeutic distinctions. Ann Pharmacother 1994;28:1359–69.
44. Bristol-Myers Squibb Co. Serzone (nefazodone) package insert. Princeton, NJ; 1997.
45. Solvay Pharmaceuticals. Luvox (fluvoxamine) package insert. Marietta, GA; 1997.
46. Robinson DS, Roberts DL, Smith JM, et al. The safety profile of nefazodone. J Clin Psychiatry 1996;57(suppl 2):31–8.
47. Pfizer, Inc. Zoloft (sertraline) package insert. Wilkes-Barre, PA; 1997.
48. Morton WA, Sonne SC, Verga MA. Venlafaxine: a structurally unique and novel antidepressant. Ann Pharmacother 1995;29:387–95.
49. Kupferschmidt HT, Riem H, Ziegler WH, Meier PJ, Krahenbuhl S. Interaction between grapefruit juice and midazolam in humans. Clin Pharmacol Ther 1995;58:20–8.
50. Fuhr U, Dummert AL. The fate of naringin in humans: a key to grapefruit juice-drug interactions: Clin Pharmacol Ther 1995;58:365–73.
51. Hollander AMJ, van Rooij J, Lentjes EGWM, et al. The effect of grapefruit juice on cyclosporine and prednisone metabolism in transplant patients. Clin Pharmacol Ther 1995;57:318–24.
52. Spence JD. Drug interactions with grapefruit: whose responsibility is it to warn the public? Clin Pharmacol Ther 1997;61:395–400.
53. Edwards DJ, Bernier SM. Naringin and naringenin are not

the primary CYP3A inhibitors in grapefruit juice. Life Sci 1996;59:1025–30.
54. Edwards DJ, Bellevue FH, Woster PM. Identification of 6,7-dihydroxybergamottin, a cytochrome P450 inhibitor, in grapefruit juice. Drug Metab Dispos 1996;24:1287–90.
55. Abramowicz M, ed. Grapefruit juice interactions with drugs. Med Lett 1995;37:73–4.
56. Benton RE, Honig PK, Zamani K, Cantilena LR, Woosley RL. Grapefruit juice alters terfenadine pharmacokinetics, resulting in prolongation of repolarization on the electrocardiogram. Clin Pharmacol Ther 1996;59:383–8.
57. Rau SE, Bend JR, Arnold MO, Tran LT, Spence JD, Bailey DG. Grapefruit juice-terfenadine single-dose interaction: magnitude, mechanism, and relevance. Clin Pharmacol Ther 1997;61:401–9.
58. Anonymous. Hismanal. FDA Med Bull 1996;269(2):4.
59. Centers for Disease Control and Prevention. Clinical update: impact of HIV protease inhibitors on the treatment of HIV-infected tuberculosis patients with rifampin. MMWR 1996;45(42):921–5.
60. Abbott Laboratories. Norvir (ritonavir) package insert. Abbott Park, IL; 1997.
61. Roche Laboratories. Invirase (saquinavir) package insert. Nutley, NJ; 1997.
62. Merck, Sharp & Dohme. Crixivan (indinavir) package insert. West Point, PA; 1997.
63. Agouron Pharmaceuticals, Inc. Viracept (nelfinavir) package insert. La Jolla, CA; 1997.
64. Humphrey PPA. Letter. BMJ 1992;305:1019.
65. Gugler R, Jensen JC. Omeprazole inhibits oxidative drug metabolism. Studies with diazepam and phenytoin in vivo and 7-ethoxycoumarin in vitro. Gastroenterology 1985;89: 1235–41.
66. Maton PN. Omeprazole. N Engl J Med 1991;324:965–75.
67. Blum RA. Lansoprazole and omeprazole in the treatment of acid peptic disorders. Am J Health-Syst Pharm 1996; 53:1401–15.
68. Anderson T. Omeprazole drug interaction studies. Clin Pharmacokinet 1991;21:195–212.
69. Darlington MR. Hypoprothrombinemia induced by warfarin sodium and cisapride. Am J Health-Syst Pharm 1997;54:320–1.
70. Maurer PM, Bartkowski RR. Drug interactions of clinical significance with opioid analgesics. Drug Saf 1993;8:30–48.
71. Kroboth P, Folan M, Lush R, et al. Coadministration of nefazodone and benzodiazepines. II. Pharmacodynamic assessment. Abstract presented at the meeting of the American Society for Clinical Pharmacology and Therapeutics, New Orleans, March 30–April 1, 1994.
72. Greene DS, Dockens RC, Salazar DE, Barbhaiya RH. Coadministration of nefazodone and benzodiazepines. I. Pharmacokinetic assessment. Abstract presented at the meeting of the American Society for Clinical Pharmacology and Therapeutics, New Orleans, March 30–April 1, 1994.
73. Barbhaiya RH, Shukla UA, Kroboth PD, Greene DS. Coadministration of nefazodone and benzodiazepines. II. A pharmacokinetic interaction study with triazolam. J Clin Psychopharmacol 1995;15:320–6.
74. Greene DS, Salazar DE, Dockens RC, Kroboth P, Barbhaiya RH. Coadministration of nefazodone and benzodiazepines. III. A pharmacokinetic interaction study with alprazolam. J Clin Psychopharmacol 1995;15:399–408.
75. Lurila H, Olkkola KT, Neuvonen PJ. Lack of interaction of erythromycin with temazepam. Ther Drug Monit 1994;16:548–51.
76. Ahonen J, Olkkola KT, Neuvonen PJ. Lack of effect of antimycotic itraconazole on the pharmacokinetics or pharmacodynamics of temazepam. Ther Drug Monit 1996;18:124–7.
77. Kharasch ED, Thummel KE. Human alfentanil metabolism by cytochrome P450 3A3/4. An explanation for interindividual variability in alfentanil clearance. Anesth Analg 1993;76:1033–9.
78. Backman JT, Olkkola, KT, Neuvonen PJ. Rifampin drastically reduces plasma concentrations and effects of oral midazolam. Clin Pharmacol Ther 1996;59:7–13.
79. Villikka K, Kivisto KT, Backman JT, Olkkola KT, Neuvonen PJ. Triazolam is ineffective in patients taking rifampin. Clin Pharmacol Ther 1997;61:8–14.
80. Bailey DG, Arnold MO, Strong A, Munoz C, Spence JD. Effect of grapefruit juice naringin on nisoldipine pharmacokinetics. Clin Pharmacol Ther 1993;54:589–94.
81. Bailey DG, Spence JD, Numoz C, Arnold JMO. Interaction of citrus juice with felodipine and nifedipine. Lancet 1991;337:268–9.
82. Leidholm H, Nordine G. Erythromycin-felodipine interaction [letter]. Drug Intell Clin Pharm 1991;25:1007–8.
83. Bailey DG, Bend JR, Arnold MO, et al. Erythromycin-felodipine interaction: magnitude, mechanism, and comparison with grapefruit juice. Clin Pharmacol Ther 1996;60:25–33.
84. Neuvonen P, Raimo S. Itraconazole interacts with felodipine. J Am Acad Dermatol 1995;33:134–5.
85. Tailor S, Gupta A, Walker S, et al. Peripheral edema due to nifedipine-itraconazole interaction: a case report. Arch Dermatol 1996;132:350–2.
86. Jalava KM, Olkkola KT, Neuvonen PJ. Itraconazole greatly increases plasma concentrations and effects of felodipine. Clin Pharmacol Ther 1997;61:410–15.
87. Bradford RH, Shear CL, Chremos AN, et al. Expanded clinical evaluation of lovastatin (EXCEL) study results. I. Efficacy in modifying plasma lipoproteins and adverse event profile in 8245 patients with moderate hypercholesterolemia. Arch Intern Med 1991;151:43–9.
88. Mauro VF. Clinical pharmacokinetics and practical applications of simvastatin. Clin Pharmacokinet 1993;24:195–202.
89. Illingworth DR, Tobert JA. A review of clinical trials comparing HMG-CoA reductase inhibitors. Clin Ther 1994;16:366–85.
90. Neuvonen PJ, Jalava K. Itraconazole drastically increases plasma concentrations of lovastatin and lovastatin acid. Clin Pharmacol Ther 1996;60:54–61.
91. Lees RS, Lees AM. Rhabdomyolysis from the coadministration of lovastatin and the antifungal agent itraconazole. N Engl J Med 1995;333:664–5.
92. Ayanian JZ, Ruchs CS, Stone RM. Lovastatin and rhabdomyolysis [letter]. Ann Intern Med 1988;109:682–3.
93. Corpier CL, Jones PH, Suke WN, et al. Rhabdomyolysis and renal injury with lovastatin use. Report of two cases in cardiac transplant recipients. JAMA 1988;260:239–41.
94. Spach DH, Bauwens JE, Clark CD, et al. Rhabdomyolysis associated with lovastatin and erythromycin use. West J Med 1991;154:213–15.
95. Fromm MF, Busse D, Kroemer HK, Eichelbaum M. Differential induction of prehepatic and hepatic metabolism of verapamil by rifampin. Hepatology 1996;24:796–801.
96. Want RW, Liu L, Cheng H. Identification of human liver cytochrome p450 isoforms involved in the in vitro metabolism of cyclobenzaprine. Drug Metab Dispos 1996;24:786–91.
97. Kivisto KT, Kroemoer HK, Eichelbaum M. The role of human cytochrome P450 enzymes in the metabolism of anticancer agents: implications for drug interactions. Br J Clin Pharmacol 1995;40:523–30.
98. Bartlett NL, Lum BL, Fisher GA, et al. Phase I trial of doxorubicin with cyclosporine as a modulator of multidrug resistance. J Clin Oncol 1994;12:835–42.
99. Rushing DA, Raber SR, Rodvold KA, Piscitelli SC, Plank GS, Tweksbury DA. The effects of cyclosporine on the pharmacokinetics of doxorubicin in patients with small cell lung cancer. Cancer 1994;74:834–41.
100. Berg SL, Cowan KH, Balis FM, et al. Pharmacokinetics of taxol and doxorubicin administered alone and in combination by continuous 72-hour infusion. J Natl Cancer Inst 1994;86:143–5.

101. Zhou-Pan XR, Serree E, Zhou XJ, et al. Involvement of human liver cytochrome P450 3A in vinblastine metabolism: drug interactions. Cancer Res 1993;53:5121–6.
102. Fedeli L, Colozza M, Boschette E, et al. Pharmacokinetics of vincristine in cancer patients treated with nifedipine. Cancer 1989;64:1805–11.
103. Buggia I, Zecca M, Alessandrino EP, et al. Itraconazole can increase systemic exposure to busulfan in patients given bone marrow transplantation. Anticancer Res 1996;16:2083–8.
104. Jacolot J, Simon I, Dreano Y, Beaune P, Riche C, Berthou J. Identification of the cytochrome P450 IIIA family as the enzymes involved in the N-demethylation of tamoxifen in human liver microsomes. Biochem Pharmacol 1991;41: 1911–19.
105. Gerberding JL. Prophylaxis for occupational exposure to HIV. Ann Intern Med 1996;125:497–501.
106. Abramowicz M, ed. New drugs for HIV infection. Med Lett 1996;38:357.
107. Liao JS, Reiss WG. Drug-cytokine interactions: focus on cyclosporine. Pharmacotherapy 1996;16:401–8.
108. Yee GC, McGuire TR. Pharmacokinetic drug interactions with cyclosporine. I. Clin Pharmacokinet 1990;19:319–32.
109. Yee GC, McGuire TR. Pharmacokinetic drug interactions with cyclosporine. II. Clin Pharmacokinet 1990;19:400–15.
110. First MR, Schroeder TJ, Michael A, Hariharan S, Weiskittel P, Alexander JW. Cyclosporine-ketoconazole interactions. Long-term follow-up and preliminary results of a randomized trial. Transplantation. 1993;55:1000–4.
111. Gomez DY, Wacher VJ, Tomlanovich SJ, Hebert MF, Benet LZ. The effects of ketoconazole on the intestinal metabolism and bioavailability of cyclosporine. Clin Pharmacol Ther 1995;58:15–19.
112. Shennib H, Auger JL. Diltiazem improves cyclosporine dosage in cystic fibrosis lung transplant recipients. J Heart Lung Transplant 1994;10:292–6.
113. McLellan RA, Drobitch RK, McLellan H, Acott PD, Crocker JFS, Renton KW. Norfloxacin interferes with cyclosporine disposition in pediatric patients undergoing renal transplantation. Clin Pharmacol Ther 1995;58:322–7.
114. Venkatesan K. Pharmacokinetic drug interactions with rifampicin. Clin Pharmacokinet 1992;22:47–65.
115. Bolt HM. Interactions between clinically used drugs and oral contraceptives. Environ Health Perspect 1994;102(suppl 9):35–8.
116. Brooks SM, Werk EE, Ackerman SJ, Sullivan I, Thrasher K. Adverse effects of phenobarbital on corticosteroid metabolism in patients with bronchial asthma. N Engl J Med 1972;286: 1125.
117. Dickey RM, ed. Managing contraceptive pill patients, 8th ed. Durant, OK: EMIS, Inc., 1994.
118. Abramowicz M, ed. Terbinafine for onychomycosis. Med Lett 1996;38:72–4.
119. Oberg K, Bauman JL. QT interval prolongation and torsades de pointes due to erythromycin lactobionate. Pharmacotherapy 1995;15:687–92.
120. Levy RH. Cytochrome P450 isoenzyme and antiepileptic drug interactions. Epilepsia 1995;36(suppl 5):S8–13.
121. Fischer V, Vogels B, Maurer G, Tynes RE. The antipsychotic clozapine is metabolized by the polymorphic human microsomal and recombinant cytochrome P450 2D6. J Pharmacol Exp Ther 1992;260:1355–60.
122. Brosen K. Are pharmacokinetic drug interactions with the SSRIs an issue? Int Clin Psychopharmacol 1996;11(suppl 1):23–7.
123. Otton SV, Wu D, Joffe RT, Cheung SW, Sellers EM. Inhibition by fluoxetine of cytochrome P450 2D6 activity. Clin Pharmacol Ther 1993;53:401–9.
124. Vandel S, Bertschy G, Bonin B, et al. Tricyclic antidepressant plasma levels after fluoxetine addition. Neuropsychobiology 1992;25:202–7.
125. Piscitelli SC, Flexner C, Minor JR, Polis MA, Masur H. Drug interactions in patients infected with human immunodeficiency virus. Clin Infect Dis 1996;23:685–93.
126. Lischke V, Behne M, Doelken P, et al. Droperidol causes a dose-dependent prolongation of the QT interval. Anesth Analg 1994;79:983–6.
127. Merck & Co., Inc. Prescribing information. Flexeril (cyclobenzaprine). West Point, PA; 1996.
128. Pollak PT, Sketris IS, MacKenzie SL, Hewlett TJ. Delirium probably induced by clarithromycin in a patient receiving fluoxetine. Ann Pharmacother 1995;29:486–8.
129. Brosen K, Gram LF. Quinidine inhibits the 2-hydroxylation of imipramine and desipramine but not the demethylation of imipramine. Eur J Clin Pharmacol 1989;37:155–60.
130. Kreek MJ, Garfield JW, Gutjahr CL, Guisi LM. Rifampin-induced methadone withdrawal. N Engl J Med 1976;294: 1104–6.
131. Upton RA. Pharmacokinetic interactions between theophylline and other medication. I. Clin Pharmacokinet 1991;20:66–80.
132. Wagner W, Vause EW. Fluvoxamine: a review of global drug-drug interactions data. Clin Pharmacokinet 1995;29:26–32.
133. Pollak PT, Slayter KL. Reduced serum theophylline concentrations after discontinuation of azithromycin: evidence for an unusual interactions. Pharmacotherapy 1997;17:827–9.
134. Grasela TH, Dreis MW. An evaluation of the quinolone-theophylline drug interaction using the Food and Drug Administration spontaneous reporting system. Arch Intern Med 1992;152:617–21.
135. Borcherding SM, Stevens R, Nichols RA, Corley CR, Self T. Quinolones: a practical review of clinical uses, dosing considerations, and drug interactions. J Fam Pract 1996;42:69–78.
136. Brouwers JR. Drug interactions with quinolone antibacterials. Drug Saf 1992;4:268–81.
137. Fuhr U, Maier A, Keller A, Steinijans VW, Sauter R, Staib AH. Lacking effect of grapefruit juice on theophylline pharmacokinetics. Int J Clin Pharmacol Ther 1995;33:311–14.
138. Bianco TM, Bussey HI, Farnett LE, et al. Potential warfarin-ciprofloxacin interaction in patients receiving long-term anticoagulation. Pharmacotherapy 1992;12:435–9.
139. Toon S, Hopkins KJ, Garstang FM, Arrons L, Sedman A, Rowland M. Enoxacin-warfarin interaction: pharmacokinetic and stereochemical aspects. Clin Pharmacol Ther 1987;42: 33–41.
140. Mitchell AS, Holland JT. Fluconazole and phenytoin: a predictable interactions. Br Med J 1989;298:1315.
141. Ortho Pharmaceutical Corporation. Topamax (topiramate) package insert. Raritan, NJ; 1997.
142. Harder S, Thurmann P. Clinically important drug interactions with anticoagulants. An update. Clin Pharmacokinet 1996;30:416–44.
143. Landes BD, Petite JP, Flouvat B. Clinical pharmacokinetics of lansoprazole. Clin Pharmacokinet 1995;28:458–70.

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A further interaction study of quinine with clinically important drugs by human liver microsomes: determinations of inhibition constant (K_i) and type of inhibition

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SUMMARY

Our previous study showed that several drugs inhibited quinine 3-hydroxylation, a cytochrome P450 (CYP) 3A4-mediated reaction, *in vitro*. In this extended study, 13 drugs were selected and tested by human liver microsomes in order to further determine their respective inhibition constant (K_i) and type of inhibition. According to the apparent K_i values, the inhibitory rank order of these tested drugs was as follows: ketoconazole > doxycycline > omeprazole > tetracycline > troleandomycin (with pre-incubation) > primaquine > troleandomycin (without pre-incubation) > nifedipine > erythromycin > verapamil > oleandomycin > diltiazem > cimetidine > hydralazine. Among these drugs, doxycycline, tetracycline, ketoconazole, nifedipine and hydralazine were judged as mixed inhibitors; whereas, the remaining other drugs tested were judged as competitive inhibitors. When the plasma/serum concentrations possibly attained after their usual therapeutic doses were taken into account, tetracycline, doxycycline, omeprazole, ketoconazole, nifedipine, troleandomycin and erythromycin are likely to be inhibitors of quinine metabolism in patients when these drugs are co-administrated with quinine.

INTRODUCTION

Many consider malaria to be the most important infectious disease in the world. Quinine is one of important antimalarial drug recommended for the treatment of chloroquine-resistant *Plasmodium falciparum* and/or complicated malaria. It is also used in non-malaria countries as treatment for leg cramps (1). Two recent clinical trials have shown that quinine is as effective as artemether, a promising antimalarial (2), both in children with cerebral malaria (3) and in adults with severe falciparum malaria (4). Thus, quinine is considered to be one of the most effective drugs for the treatment of malaria.

The primary route of elimination of quinine in humans is hepatic metabolism with less than 20% of the drug excreted unchanged in urine (5–7). Recently, several

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studies indicated that the formation of 3-hydroxyquinine from quinine is the major metabolic pathway (8,9) and the 3-hydroxylation is catalysed mainly by CYP3A4 (10,11) and to a minor extent by CYP2C19 (10) in human liver microsomes. In clinical practice, many inhibitors and/or inducers of CYP3A4 (12–14) are widely used and quinine is also co-administered with other antimalarials for the treatment and/or chemoprophylaxis of malaria (5,6,15,16). In addition, it has been know that quinine possess a relatively low therapeutic index with some adverse reactions such as cinchonism and cardiac arrhythmias (5,6,16). Thus, quinine–drug interactions appear to be of clinical importance. Indeed, a recent *in vitro* study (17) has shown that several antimalarials (e.g. primaquine, doxycycline and tetracycline) and non-antimalarials (e.g. ketoconazole, troleandomycin and omeprazole) inhibited quinine 3-hydroxylation in a concentration-dependent manner by human liver microsomes. Although the authors estimated the IC_{50} (i.e. a 50% inhibition of 3-hydroxyquinine formation, compared with the control) values of those inhibitors/substrates, and predicted the possible *in vivo* drug interactions between quinine and those related drugs according to the calculated IC_{50} values as well as their normal therapeutic plasma concentrations in patients, the inhibition constant (K_i) and the type of inhibition were not determined. However, it is considered that the K_i value and the type of inhibition would be more helpful than IC_{50} values for accurately predicting the *in vivo* drug–drug interactions. This is because the IC_{50} values vary with the substrate concentration and in many cases the IC_{50} values are not equal, although sometimes close, to the K_i values.

Thus, the aims of the present extended *in vitro* study were: (i) to determine the K_i values and the type of inhibition of several antimalarial and non-antimalarial drugs that showed a relatively potent inhibition on quinine 3-hydroxylation in our previous study (17); and (ii) to re-predict the possible quinine–drug interactions *in vivo* by comparison of the estimated K_i values obtained from this *in vitro* study with the free concentrations in plasma/liver of those drugs.

MATERIALS AND METHODS

Drugs and chemicals

Synthetic 3-hydroxyquinine was a generous gift from Dr Winstanley (University of Liverpool, Liverpool, UK). Primaquine, doxycycline, verapamil, ketoconazole, troleandomycin (TAO), cimetidine, erythromycin, nifedipine, diltiazem, oleandomycin and hydralazine were purchased from Sigma Chemical Co. (St Louis, MO, USA), omeprazole from Fujisawa-Astra (Osaka, Japan), tetracycline, acetonitrile, methanol and other reagents of analytical grade from Wako Pure Chemical Industries Ltd (Osaka, Japan). $NADP^+$, glucose-6-phosphate and glucose-6-phosphate dehydrogenase were obtained from Oriental Yeast (Tokyo, Japan).

Enzyme preparation

Six histologically normal liver samples were obtained from Japanese patients undergoing a partial hepatectomy as described previously (17,18). Liver microsomes were prepared by the classical differential centrifugation technique (18,19). After the determination of microsomal protein by the method of Lowry *et al* (20), the individual microsomal samples were aliquoted and stored at –80°C until used.

Enzyme assays

The basic incubation medium contained 0.05–0.1 mg/ml human liver microsomes, 0.5 mM $NADP^+$, 2.0 mM glucose-6-phosphate, 1 IU/ml glucose-6-phosphate dehydrogenase, 4 mM $MgCl_2$, 0.1 mM EDTA, 100 mM potassium buffer (pH 7.4) and different concentrations of quinine and inhibitors in a final volume of 250 µl. All the reactions, except for TAO, were initiated by addition of the NADPH-generating system without pre-incubation, and the mixture was incubated at 37°C in a shaking water bath for 15 min. The reactions were initiated for TAO in two different ways: incubation mixture containing TAO was once pre-incubated in the presence of the NADPH-generating system at 37°C for 15 min and then the reactions were initiated by addition of the substrate quinine. The reaction was also initiated without pre-incubation by addition of the NADPH-generating system with the incubation mixture containing quinine and TAO simultaneously, and the mixture was incubated at 37°C for 15 min. After the reaction was stopped by addition of 500 µl of cold methanol, the mixture was centrifuged at 1500 *g* for 10 min, and 30 µl supernatant was injected onto a high-performance liquid chromatography (HPLC) system as described below.

3-Hydroxyquinine was examined in the incubation mixture by the HPLC method using fluorometric detection, according to a published method (9), with minor modifications as employed in our recent study (17). Briefly, the HPLC system consisted of a model L7100 pump (Hitachi Ltd, Tokyo, Japan), a model L-7200 autosampler (Hitachi), a model D-7500 integrator

(Hitachi) and a 2 x 100 mm reversed-phase C_{18} column (Shandon, London, UK). The mobile phase consisted of a 40/60 (v/v) mixture of acetonitrile and 0.05 M sodium phosphate buffer, containing 10 mM sodium dodecyl sulfate and 0.1 mM tetrabutylammonium bromide. The pH of the mobile phase was finally adjusted to 2.1, and the mobile phase was delivered at a flow rate of 0.5 ml/min, Inter- and intra-assay coefficients of variation for each procedure ($n = 6$) were < 10%, and the lowest limits of detection for both 3-hydroxyquinine and quinine, defined as the lowest concentration with a signal-to-noise ratio of 10, were 5 ng/ml.

Inhibition study on quinine 3-hydroxylation by selected drugs

Those drugs which showed an inhibitory effect for quinine 3-hydroxylation as observed in our previous experiment (17) were selected for this study. All the tested drugs were dissolved in methanol. To identify the respective K_i values and the type of inhibition, different concentrations of quinine, range 25–400 µM were used and the various inhibitor/substrate concentrations were chosen range 0.001–300 µM. An aliquot (50 µl) of each drug dissolved in methanol was evaporated to dryness before addition of the other reaction constituents. Pooled microsomal samples from six different individuals were used in this study since the microsomal protein available was not sufficient to test all the drugs under identical and reproducible conditions.

The apparent kinetic parameters (i.e. K_m and V_{max}) were estimated initially by use of the non-linear least-squares regression analysis program, MULTI (21), after the graphic data representation of Lineweaver-Burk plots. The apparent K_i values were determined by the secondary plots (i.e. K_m/V_{max} ratio as a function of the inhibitor concentration). This kinetic analysis approach was attempted because certain of our data did not appear to fit simple inhibition models (e.g. Dixon plots, not shown). In addition to the visualised data fitting by the Lineweaver-Burk plots, the inhibition data were evaluated for competitive, non-competitive, uncompetitive and mixed-type patterns according to conventionally proposed criteria (22) as has been done by Bourrie *et al* (23).

Table I: Characteristics of the inhibition of quinine 3-hydroxylation by various probes selected for the present *in vitro* human liver microsomal study

Drugs	Apparent K_i (µM)	Type of inhibition
Primaquine	14	Competitive
Doxycycline	1.1	Mixed
Tetracycline	7.3	Mixed
Verapamil	42	Competitive
Omeprazole	4.5	Competitive
Ketoconazole	0.016	Mixed
TAO*	17.7	Competitive
TAO**	7.7	Competitive
Cimetidine	61	Competitive
Erythromycin	39	Competitive
Nifedipine	24	Mixed
Diltiazem	55	Competitive
Oleandomycin	47	Competitive
Hydralazine	105	Mixed

Abbreviations: TAO, troleandomycin; K_i, inhibition constant.
*TAO was not pre-incubated with microsomes in the presence of the NADPH generating system for 15 min.
**TAO was pre-incubated with microsomes in the presence of the NADPH generating system for 15 min.
The apparent K_i values are derived from the secondary plots (i.e. K_m/V_{max} ratio versus the inhibitor concentration) of each drug using a pooled microsomal sample obtained from 6 different human livers.

RESULTS

Chromatograms and assessment of incubation conditions

Using the chromatographic conditions described above, none of the drugs tested gave rise to any chromatographic peaks interfering with 3-hydroxyquinine and quinine. Preliminary studies revealed that the 3-hydroxylation of quinine with human liver microsomes was linear with regard to the incubation time from 5–60 min when 100 µM (around the apparent K_m) of quinine was incubated with microsomes equivalent to 0.1 mg of protein/ml. A linear relationship was also observed between the rate of metabolite production in 15 min and protein concentration for up to 0.25 mg/ml. Accordingly, the subsequent kinetic and inhibition studies were performed with a 15 min incubation time and a microsomal protein content of 0.05–0.1 mg/ml.

Inhibition experiments

Thirteen drugs that showed an inhibition ($IC_{50} < 200$ µM) of quinine 3-hydroxylation in our previous *in vitro* study (17) were used in this extensive study. All 13 drugs inhibited the microsomal metabolism of quinine in a concentration-dependent manner, but the magnitude of the inhibition differed among them (Table I). The inhibitory rank order of these drugs was as follows: ketoconazole > doxycycline > omeprazole > tetracycline > TAO (with pre-incubation) >

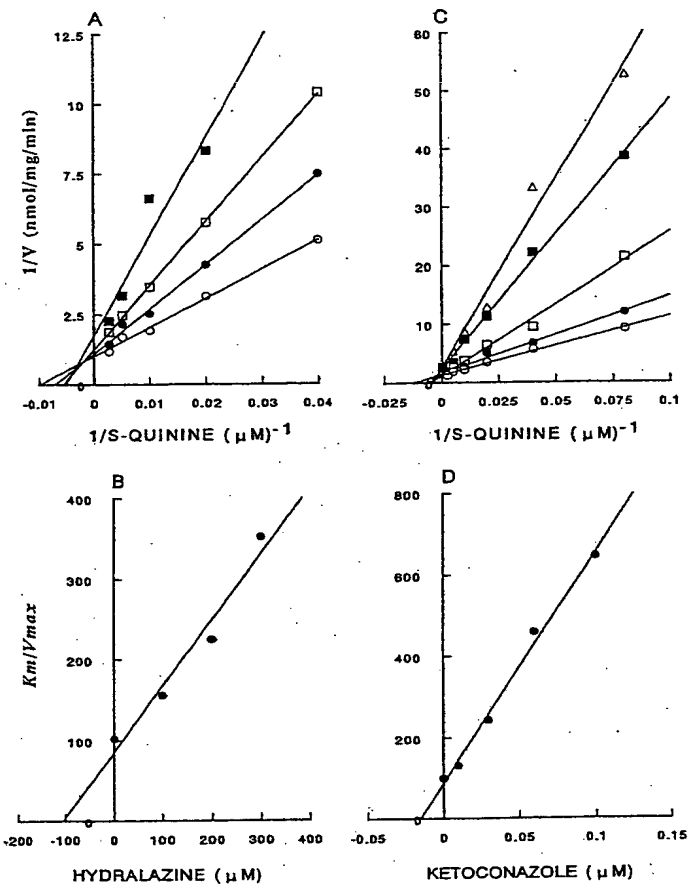

Fig. 1: Representative Lineweaver-Burk plots (A,C) and the corresponding secondary plots (B,D) for the inhibition of quinine 3-hydroxylation by hydralazine (A,B): open circles, 0 μM; filled circles, 100 μM; open squares, 200 μM; and filled squares, 300 μM in (A) and by ketoconazole (C,D), open circles, 0 μM; filled circles, 0.01 μM; open squares, 0.03 μM; filled squares, 0.06 μM; and open triangles 0.1 μM in (C). The data were obtained using a pooled liver microsomal sample obtained from 6 different livers, and judged to exhibit a mixed-type inhibition for quinine 3-hydroxylation,

primaquine > TAO (without pre-incubation) > nifedipine > erythromycin > verapamil > oleandomycin > diltiazem > cimetidine > hydralazine according to the respective apparent K_i values (Table I).

The representative Lineweaver-Burk and the corresponding secondary plots for the inhibitory effect of hydralazine and ketoconazole on quinine 3-hydroxylation by human liver microsomal samples obtained from pooled six different livers are shown in Figure 1. Metabolic constants for quinine 3-hydroxylation were 100 μM for K_m and 0.98 nmol/min/mg for V_{max}. With increasing hydralazine or ketoconazole concentrations, a concentration-dependent increase in the apparent K_m values as well as a decrease in the V_{max} values. This is consistent with a mixed-type inhibition (22,23). The apparent K_i values for hydralazine and ketoconazole were estimated (K_m/V_{max} as a function of inhibitor concentration) at 105 and 0.01 6 μM, respectively (Fig. 1B,D & Table I). Similarly, doxycycline, tetracycline and nifedipine were judged to exhibit a mixed-type inhibition for quinine 3-hydroxylation.

Quinine 3-hydroxylation is catalysed mainly by CYP3A4 *in vitro* (10,11,17). TAO is a mechanism-based inhibitor of this CYP isoform and requires an NADPH-dependent complexion for inactivation (24). Thus, experiments were performed using two different protocols. First, the inhibitory effect of TAO was investigated after the co-incubation of quinine and TAO with human liver microsomes. Under this *in vitro* condition, the apparent K_m and V_{max} values for quinine 3-hydroxylation were 120 μM and 1.1 nmol/min/mg, respectively. With increasing TAO concentrations, a concentration-dependent inhibition of quinine 3-hydroxylation was observed. It was characterised by an increase in the apparent K_m value, whereas the V_{max} value was not substantially affected. This is consistent with a competitive-type inhibition between quinine and TAO at the level of the active site. The apparent K_i value for TAO was estimated at 17.7 μM (Table I). This competitive-type inhibition was also observed for primaquine, verapamil, omeprazole, cimetidine, erythromycin, diltiazem and oleandomycin when these drugs were co-incubated with quinine simultaneously (Table I). The two representative Lineweaver-Burk and corresponding secondary plots for the competitive inhibition effects of cimetidine and diltiazem on quinine 3-hydroxylation are shown in Figure 2.

In the second protocol, human liver microsomes were pre-incubated for 15 min with TAO in the presence of the NADPH-generating system. The pre-incubated microsomes were then incubated with increasing quinine concentrations and the rate of quinine 3-hydroxylation was evaluated. The apparent K_m and V_{max} values for quinine 3-hydroxylation were 100 μM and 0.9 nmol/min/mg, respectively. After pre-incubation of microsomes with increasing TAO concentrations, a concentration-dependent inhibition of quinine 3-hydroxylation was observed, with an increase in K_m and no appreciable change in V_{max}. This is also consistent with a competitive-type inhibition (22,23). The mean K_i value for TAO obtained under this *in vitro* condition was calculated to be 7.7 μM (Table I).

DISCUSSION

Our previous study (17) showed that several antimalarial and non-antimalarial drugs inhibited the 3-hydroxylation

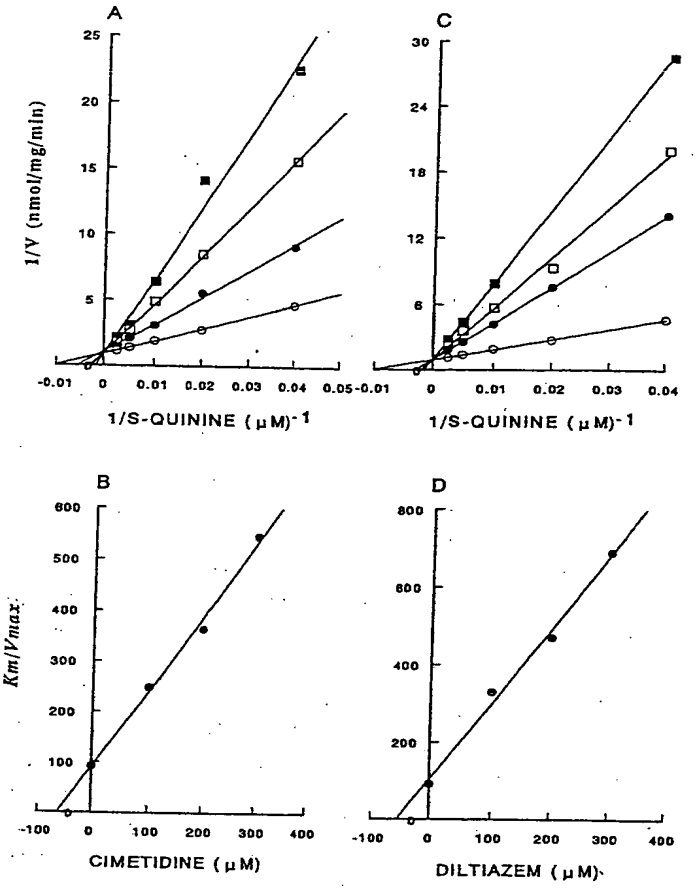

Fig. 2: Representative Lineweaver-Burk plots (**A,C**) and the corresponding secondary plots (**B,D**) for the inhibition of quinine 3-hydroxylation by cimetidine (**A,B**): open circles, 0 μM; filled circles, 100 μM; open squares, 200 μM; and filled squares 300 μM in (**A**); and by diltiazem (**C,D**), open circles, 0 μM; filled circles, 100 μM; open squares, 200 μM ;and filled squares, 300 μM in (**C**). The data were obtained using a pooled liver microsomal sample obtained from 6 different livers, and judged to exhibit a competitive-type inhibition for quinine 3-hydroxylation.

of quinine in human liver microsomes. In this extended *in vitro* microsomal study, we determined the respective apparent K_i values and the type of inhibition for each of the drugs tested. We observed that antimalarial drugs, doxycycline, primaquine and tetracycline, were relatively potent inhibitors for quinine 3-hydroxylation in human liver microsomes with apparent K_i values of 1.1, 7.3 and 14 μM, respectively (Table I). Thus, on a theoretical basis, a drug interaction may occur between quinine and these drugs *in vivo*. Nevertheless, such a prediction must be confirmed in the clinical situation by a kinetic study on quinine in patients with malaria given quinine concurrently with other antimalarials. This is because an *in vivo* drug interaction is dependent on blood or, more importantly, hepatic tissue drug concentrations. Moreover, several other factors (e.g. protein binding) are excluded from an *in vitro* experiment. With the limitations as cited above, we wish to discuss on our findings as below.

Primaquine and verapamil may not have any clinical significance since their maximum serum concentrations (0.4 and 0.8 μM, respectively) attained after their usual doses (25) are much lower than their apparent K_i values (1 4 and 42 μM, respectively; Table I). On the other hand, doxycycline and tetracycline reach serum concentrations of about 4 and 8 μM after normal doses (26), which are close their apparent K_i values (1.1 and 7.3 μM, respectively; Table I). Hence, the possibility cannot totally be ruled out that these antibiotics may cause an interaction when co-administered with quinine *in vivo*. Indeed, Karbwang *et al* (27) have observed a pharmacokinetic interaction between doxycycline and quinine (i.e. plasma levels of quinine were elevated by doxycycline), although another clinical study (28) failed to detect an interaction in patients with acute falciparum malaria.

Among the drugs listed in Table I, Ca^{2+}-antagonists (verapamil, nifedipine and diltiazem), macrolide antibiotics (TAO, erythromycin and oleandomycin), ketoconazole, omeprazole and cimetidine are inhibitor/ substrates of CYP3A4 (12–14,29,30). Although to date no information is available on the involvement of CYP isoform(s) in the metabolism of primaquine, doxycycline and tetracycline in humans, our data suggest that they might be substrates or inhibitors of CYP3A4. Supporting this, Na Bangchang *et al* (31) have indicated that ketoconazole, a CYP3A4 inhibitor (12,13), was a potent inhibitor of primaquine metabolism in human liver microsomes. However, the authors employed a > 25 μM concentration of ketoconazole, and the IC_{50} and apparent K_i values yielded by this inhibitor for the primaquine metabolism were 15 and 6.7 μM, respectively. At such concentrations, ketoconazole inhibits several CYP isoforms including CYP3A4 (32), 2C (33,34) and 1A2 (35). Studies (23,36) have shown the specificity of ketoconazole toward CYP3A when used at a low concentration (< 1 μM). Therefore, from the data of Na Bangchang *et al* (31), no definite conclusion can be drawn that primaquine is a substrate or inhibitor of CYP3A4, although our data suggest this possibility.

Different inhibitory effects of TAO were observed with and without pre-incubation of the microsomal samples (Table I). Pre-incubation of microsomes with TAO for 15 min gave an apparent K_i value of 7.7 μM. Without pre-incubation, the apparent K_i value was 17.7 μM (Table I). A considerable loss of quinine 3-hydroxylase activity occurs

during the 15 min pre-incubation with TAO, suggesting that the mechanism-based inactivation is important for the inhibitory effects. Thus, the observation that TAO is a mechanism-based inhibitor of quinine 3-hydroxylation, mediated dominantly by CYP3A4 (10,11), is in agreement with the results reported by Watkins *et al* (24).

Omeprazole, a substrate of CYP2C19 (14,18,29), inhibited the 3-hydroxylation of quinine with the apparent K_i value of 4.5 μM (Table I). This is in agreement with our recent study (10) that the 3-hydroxylation of quinine is partly catalysed by CYP2C19. However, omeprazole is also a substrate for CYP3A4 (14,29). In addition, the content of CYP3A is highest in human liver microsomes (about 30% of the total CYPs) (37), whereas the content of CYP2C19 is only about 1% of the total CYPs (37). Therefore, omeprazole might inhibit the activity of CYP3A4 more than that of CYP2C19, since quinine is chiefly 3-hydroxylated by CYP3A4 (10,11). On the other hand, the apparent K_m values for omeprazole metabolism were about 8 μM via CYP2C19 and about 49 μM via CYP3A4 (29), whereas the apparent K_i value of omeprazole for quinine 3-hydroxylation was 4.5 μM (Table I). Therefore, omeprazole appears to have a greater inhibition potential for CYP3A than CYP2C19. Christians *et al* (38) have also reported that omeprazole inhibits the metabolism of an immunosuppressive macrolide, tacrolimus [a substrate of CYP3A4 (12,13)], competitively with a mean apparent K_i value of 34 μM.

Of the non-antimalarial drugs tested, some were known substrates/inhibitors of CYP3A (12–14,29,30). Therefore, it is reasonable to observe inhibition of quinine 3-hydroxylation by these drugs. Indeed, ketoconazole was a potent inhibitor of quinine metabolism (Table I), with an apparent K_i value of 0.016 μM, followed by TAO (with and without pre-incubation), nifedipine and erythromycin. Because the plasma/serum concentrations of these drugs attained after their usual therapeutic doses (25,26) are close to or higher than their respective apparent K_i values (Table I), a significant interaction may occur when these drugs are administered concomitantly with quinine *in vivo*. Other substrates/inhibitors of CYP3A4, which were weaker inhibitors (e.g. diltiazem with an apparent $K_i > 50$ μM) of quinine metabolism (Table I), may not have any significant inhibitory effects on quinine 3-hydroxylation *in vivo*. This observation is consistent with a previous study (39), indicating that various CYP3A4 substrates interact differently with the enzyme or perhaps with different isoforms. This may partly explain the observed lack of the *in vivo* correlation between some substrates.

In conclusion, although we recognise that a drug identified as an inhibitor in an *in vitro* study is not necessarily an inhibitor in the *in vivo* situation, drugs that fail to inhibit the metabolism of a substrate *in vitro* will not be inhibitors *in vivo* (38). Thus, for the antimalarial or non-antimalarial drugs, which showed inhibition of quinine metabolism *in vitro* (Table I), further assessment of the possible interactions is required in clinical studies, particularly for doxycycline, tetracycline, omeprazole, ketoconazole, TAO, nifedipine and erythromycin. When these drugs and quinine are prescribed concurrently in patients with malaria, a close monitoring of the blood (plasma or serum) concentrations of quinine may be required to avoid supratherapeutic quinine concentrations [e.g. > 20 μg/ml (6,16)] and thereby related toxicity.

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REFERENCES

1. Dyer J.R., Davis T.M.E., Giele C., Annus T., Garcia-Webb P., Robson J. (1994) : The pharmacokinetics and pharmacodynamics of quinine in the diabetic and non-diabetic elderly. Br. J. Clin. Pharmacol., 38: 205–212.
2. Hien T.T., White N.J. (1993) : Qinghaosu. Lancet, 341: 603–608.
3. Boele van Hensbroek M., Onyiprah E., Jaffar S. *et al.* (1996) : A trial of artemether or quinine in children with cerebral malaria. N. Engl. J. Med., 335: 69–75.
4. Hien T.T., Day N.P.J., Phu N.H. *et al.* (1996) : A controlled trial of artemether or quinine in Vietnamese adults with severe falciparum malaria. N. Engl. J. Med., 335: 76–83.
5. Tracy J.W., Webster L.T. (1996) : Drugs used in the chemotherapy of protozoal infections. Malaria. In: Hardman J.G., Limbird L.E., Monlinoff P.B., Ruddon R.W., Gilman A.G. (Eds) Goodman & Gilman's The Pharmacological Basis of Therapeutics, 9th Edn. New York: McGraw-Hill, 965–985.
6. White N.J. (1992) : Antimalarial pharmacokinetics and treatment regimens. Br. J. Clin. Pharmacol., 34: 1–10.
7. Krishna S., White N.J. (1996) : Pharmacokinetics of quinine, chloroquine and amodiaquine. Clin. Pharmacokinet., 30: 263–299

8. Wanwimolruk S., Wong S.M., Zhang H., Coville P.F., Walker R.J. (1995) : Metabolism of quinine in man: identification of a major metabolise, and effects of smoking and rifampicin pretreatment. J. Pharm. Pharmacol., 47: 957–963.
9. Wanwimolruk S., Wong S.M., Zhang H., Coville P.F. (1996) : Simultaneous determination of quinine and a major metabolite 3-hydroxyquinine in biological fluids by HPLC without extraction. J. Liq. Chromatogr., 19: 293–305.
10. Zhao X.J., Yokoyama H., Chiba K., Wanwimolruk S., Ishizaki T. (1996) : Identification of human cytochrome P450 isoforms involved in the 3-hydroxylation of quinine by human liver microsomes and nine recombinant human cytochromes P450. J. Pharmacol. Exp. Ther., 279: 1327–1334.
11. Zhang H., Coville P.F., Walker R.J., Miners J.O., Birkett D.J., Wanwimolruk S. (1997) : Metabolism of quinine in humans: evidence for the involvement of human CYP3A in the hepatic metabolism of quinine. Br. J. Clin. Pharmacol., 43: 245–252.
12. Wrighton S.A., Stevens J.C. (1992) : The human hepatic cytochromes P450 involved in drug metabolism. Crit. Rev. Toxicol., 22: 1–21.
13. Brockmoller J., Roots I. (1994) : Assessment of liver metabolic function: clinical implications. Clin. Pharmacokinet., 27: 216–248.
14. Andersson J. (1996) : Pharmacokinetics, metabolism and interactions of acid pump inhibitors. Focus on omeprazole, lasoprazole and pantoprazole. Clin. Pharmacokinet., 31: 9–28.
15. Bradley D. (1993) : Prophylaxis against malaria for travellers from the United Kingdom. BMJ, 306: 1247–1252.
16. White N.J. (1988) : Drug treatment and prevention of malaria. Eur. J. Clin. Pharmacol., 34: 1–14.
17. Zhao X.J., Ishizaki T. (1997) : Metabolic interactions of selected antimalarial and non-antimalarial drugs with the major pathway (3-hydroxylation) of quinine in human liver microsomes. Br. J. Clin. Pharmacol., 44: 505–511.
18. Chiba K., Kobayashi K., Manabe K., Tani M., Kamataki T., Ishizaki T. (1993) : Oxidative metabolism of omeprazole in human liver microsomes: cosegregation with S-mephenytoin 4'-hydroxylation. J. Pharmacol. Exp. Ther., 266: 52–59.
19. Echizen H., Kawasaki H., Chiba K., Tani M., Ishizaki T. (1993) : A potent inhibitory effect of erythromycin and other macrolide antibiotics on the mono-N-dealkylation metabolism of disopyramide with human liver microsomes. J. Pharmacol. Exp. Ther., 82: 61–73.
20. Lowry O.H., Rosebrough N.J., Farr A.L., Randall R.J. (1951) : Protein measurement with the Folin phenol reagent. J. Biol. Chem., 193: 265–275.
21. Yamaoka K., Tanigawara Y., Nakagawa T., Uno J. (1981) : A pharmacokinetic analysis program (MULTI) for microcomputer. J. Pharm. Dyn., 4: 879–885.
22. Segel I.H. (1975) : Behavior and analysis of rapid equilibrium and steady-state enzyme system. In: Enzyme Kinetics New York: John Wiley, 100–226.
23. Bourrie M., Meunier V., Berger Y., Fabre G. (1996) : Cytochrome P450 isoform inhibitions as a tool for the investigation of metabolic reactions catalysed by human liver microsomes. J. Pharmacol. Exp. Ther., 277: 321–332.
24. Watkins P.B., Wrighton S.A., Maure J.P. *et al.* (1985) : Identification of an inducible form of cytochrome P-450 in human liver. Proc. Natl. Acad. Sci. USA, 82: 6310–6314.
25. Dollery S.C., Boobis A.R., Burley D. *et al.* (1991): Verapamil: pp. V12–V18, nifedipine: pp. N80–N87 and primaquine: pp. P209–P218, In: Therapeutic Drugs. London: Churchill Livingston.
26. Kapusnik-Uner J.E., Sande M.A., Chambers H.F. (1996) : Antimicrobial agents. tetracycline, chloramphenicol, erythromycin, and miscellaneous antibacterial agents. In: Hardman J.G., Limbird L.E., Monlinoff P.B., Ruddon R.W., Gilman A.G. (Eds) Goodman and Gilman's The Pharmacological Basis of Therapeutics, 9th Edn. New York: McGraw-Hill, 1123–1153.
27. Karbwang J., Bangchang K.N., Thanavibul A., Wattanakoon Y., Harinasuta T. (1994) : Quinine toxicity when given with doxycycline and mefloquine. Southeast Asian J. Trop. Med. Public Health, 25: 397–400.
28. Couet W., Laroche R., Floch J.J., Istin B., Fourtillan J.B., Sauniere J.F. (1991) : Pharmacokinetics of quinine and doxycycline in patients with acute falciparum malaria: a study in Africa. Ther. Drug. Monit., 13: 496–501.
29. Andersson T., Miners J.O., Veronese M.E., Birkett D.J. (1993) : Identification of human liver cytochrome P450 isoforms mediating omeprazole metabolism. Br. J. Clin. Pharmacol., 36: 521–530.
30. Andersson T., Miners J.O., Veronese M.E., Birkett D.J. (1994) : Diazepam metabolism by human liver microsomes is mediated by both S-mephenytoin hydroxylase and CYP3A isoforms. Br. J. Clin. Pharmacol., 38: 131–137.
31. Na Bangchang K., Karbwang J., Back D.J. (1992) : Primaquine metabolism by human liver microsomes: effect of other antimalarial drugs. Biochem. Pharmacol., 44: 587–590.
32. Maurice M., Pichard L., Daujat M. *et al.* (1992) : Effects of imidazole derivatives on cytochromes P450 from human hepatocytes in primary culture. FASEB J., 6: 752–758.
33. Back D.J., Tjia J.F., Karbwang J., Colbert J. (1988) : *In vitro* inhibition studies of tolbutamide hydroxylase activity of human liver microsomes by azoles, sulphonamides and quinolines. Br. J. Clin. Pharmacol., 26: 23–29.
34. Back D.J., Stevenson P., Tjia J.F. (1989) : Comparative effects of two antimycotic agents, ketoconazole and terbinafine, on the metabolism of tolbutamide, ethinyloestradiol, cyclosporin and ethoxycoumarin by human liver microsomes *in vitro*. Br. J. Clin. Pharmacol., 28: 166–170.
35. Eugster H.P., Sengstag C. (1993) : *Saccharomyces cerevisae*: an alternative source for human liver microsomal enzymes and its use in drug interaction studies. Toxicology, 82: 61–73.
36. Newton D.J., Wang R.W., Lu A.Y.H. (1995) : Cytochrome P450 inhibitors: evaluation of specificities in the *in vitro* metabolism of therapeutic agents by human liver microsomes. Drug Metab. Dispos., 23: 154–158.
37. Shimada J., Yamazaki H., Miura M., Inui Y., Guengerich F.P. (1994) : Interindividual variations in human liver cytochrome P-450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: studies with liver microsomes of 30 Japanese and 30 Caucasians. J Pharmacol. Exp. Ther., 270: 414–423.
38. Christians U., Schmidt G., Bader A. *et al.* (1996) : Identification of drugs inhibiting the *in vitro* metabolism of tacrolimus by human liver microsomes. Br. J. Pharmacol., 41: 187–190.
39. Irshaid Y., Branch R.A., Adedoyin A. (1996) : Metabolic interactions of putative cytochrome P4503A substrates with alternative pathways of dapsone metabolism in human liver microsomes. Drug Metab. Dispos., 24: 164–171.

Simvastatin but not pravastatin is very susceptible to interaction with the CYP3A4 inhibitor itraconazole

Background: Itraconazole increases the risk of skeletal muscle toxicity of some 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors by increasing their serum concentrations. We studied possible interactions of itraconazole with simvastatin and pravastatin.

Methods: Two randomized, double-blind, two-phase crossover studies were performed with use of an identical design, one with simvastatin (study I) and one with pravastatin (study II). In both studies, 10 healthy volunteers received either 200 mg itraconazole or placebo orally once a day for 4 days. On day 4, each subject ingested a single 40 mg dose of simvastatin (study I) or pravastatin (study II). Serum concentrations of simvastatin, simvastatin acid, pravastatin, HMG-CoA reductase inhibitors, itraconazole, and hydroxyitraconazole were determined.

Results: In study I, itraconazole increased the peak serum concentrations (C_{max}) and the areas under the serum concentration–time curve [AUC(0-∞)] of simvastatin and simvastatin acid at least tenfold ($p < 0.001$). The C_{max} and AUC(0-∞) of total simvastatin acid (naive simvastatin acid plus that derived by hydrolysis of the lactone) were increased 17-fold and 19-fold ($p < 0.001$), respectively, and the half-life ($t_{1/2}$) was increased by 25% ($p < 0.05$). The AUC(0-∞) of HMG-CoA reductase inhibitors was increased fivefold ($p < 0.001$) and the C_{max} and $t_{1/2}$ were increased threefold ($p < 0.001$). In study II, itraconazole slightly increased the AUC(0-∞) and C_{max} of pravastatin, but the changes were statistically nonsignificant ($p = 0.052$ and 0.172, respectively). The $t_{1/2}$ was not altered. The AUC(0-∞) and C_{max} of HMG-CoA reductase inhibitors were increased less than twofold ($p < 0.05$ and $p = 0.063$, respectively) by itraconazole. There were no differences in the serum concentrations of itraconazole and hydroxyitraconazole between studies I and II.

Conclusions: Itraconazole greatly increased serum concentrations of simvastatin, simvastatin acid, and HMG-CoA reductase inhibitors, probably by inhibiting CYP3A-mediated metabolism, but it had only a minor effect on pravastatin. Concomitant use of potent inhibitors of CYP3A with simvastatin should be avoided or its dosage should be greatly reduced. (Clin Pharmacol Ther 1998;63:332-41.)

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Simvastatin and pravastatin are inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, and they have beneficial effects on coronary disease and mortality rates in patients with hypercholesterolemia.[1-2] Skeletal muscle toxicity is a rare side effect of HMG-CoA reductase inhibitors in monotherapy. However, an increased risk of myalgia, as well as cases of rhabdomyolysis, have been reported after the concomitant use of HMG-CoA reductase inhibitors with certain other drugs, for example, itraconazole, cyclosporine (INN, ciclosporin), and erythromycin.[3-6]

The antimycotic itraconazole greatly increases plasma concentrations of lovastatin and lovastatin acid.[7] Lovastatin and simvastatin are substrates of CYP3A, whereas the enzymes responsible for the biotransformation of pravastatin are less well known.[8-11] In any event, increased serum concentrations of simvastatin, lovastatin, and pravastatin have been observed in organ transplant patients taking

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cyclosporine.[12,13] Both itraconazole and cyclosporine are potent inhibitors of CYP3A4.[8]

The aim of this study was to characterize the effects of itraconazole on the pharmacokinetics of simvastatin and pravastatin in healthy subjects and to determine their susceptibility to interaction with inhibitors of CYP3A.

METHODS

Study design. This investigation was composed of two separate studies that had an otherwise identical design except that study I was performed with simvastatin and study II was performed with pravastatin. Both of these studies, including human experiments and chemical and data analyses, were carried out in parallel. The investigation was approved by the Ethics Committee of the Department of Clinical Pharmacology, University of Helsinki and the National Agency for Medicines (Helsinki, Finland).

Seven male and three female volunteers (age range, 19 to 29 years; weight range, 55 to 80 kg) participated in study I, and seven male and three female volunteers (age range, 19 to 23 years; weight range, 52 to 86 kg) in study II (Table I), after giving written informed consent. The subjects were ascertained to be healthy by a clinical examination and laboratory tests (e.g., blood hemoglobin, serum creatinine, alanine aminotransferase, and creatine kinase). All subjects but one (Subject 16 in study II) were nonsmokers, and none of the subjects was receiving continuous medication except for five female subjects (two in study I and three in study II) who were using oral contraceptive steroids (Table I).

In both studies, a randomized, double-blind, crossover study design in two phases was used at intervals of 4 weeks. In both studies, the volunteers took either 200 mg itraconazole (Sporanox, Janssen Pharma, Beerse, Belgium) or placebo orally at 7:30 AM for 4 days. On day 4 of study I, each subject ingested a single 40 mg dose of simvastatin (Zocor, MSD, Haarlem, Netherlands) with 150 ml water at 9:30 AM. On day 4 of study II, each subject ingested a single 40 mg dose of pravastatin (Pravachol, Bristol-Myers Squibb, Bromma, Sweden) with 150 ml water at 9:30 AM. In both studies, the subjects fasted for 2 hours before the ingestion of simvastatin or pravastatin and had a standard meal 4 hours afterward.

Blood sampling. On the days of administration of simvastatin or pravastatin, a forearm of each subject was cannulated with a plastic cannula and kept patent with an obturator. Blood was sampled into siliconized Venoject tubes (Terumo Europe, Leuven, Belgium). Timed blood samples (10 ml) were collected immediately before administration of simvastatin or pravastatin and ½, 1, 2, 3, 4, 6, 8, 12, and 24 hours after administration. Serum was separated within 30 minutes, each sample being divided into four tubes and stored at –70° C until analyzed.

Determination of simvastatin by HPLC. Serum concentrations of unchanged simvastatin (study I) were quantified by HPLC.[14] Lovastatin was used as an internal standard. The detection limit was 10 ng/ml. The between-day coefficient of variation (CV) was 8.0% (at 117 ng/ml; $n = 6$). Itraconazole did not interfere with the analytical method.

Determination of simvastatin acid and pravastatin by gas chromatography–mass spectrometry (GC-MS). Serum concentrations of total simvastatin acid[15] (that present in serum plus that derived by hydrolysis of the simvastatin lactone; study I) and pravastatin[16] (study II) were quantified by GC-MS. In short, in study I, simvastatin, simvastatin acid, and lovastatin (internal standard) were extracted from serum into methanol with use of Bond Elut C_2 solid-phase columns. After evaporation to dryness, 2 ml water and 0.5 ml of 0.05N potassium hydroxide were added and the samples were incubated at 40° C for 15 minutes to hydrolyze lactones to their acid forms (this was necessary for derivatization). Simvastatin acid and lovastatin acid were then extracted into methanol with use of C_{18} solid-phase columns. In study II, pravastatin and the internal standards (d_5-SQ-31,000 and d_5-SQ-31,906) were extracted from serum and purified with use of C_{18} solid-phase columns. For the derivatization, alpha-bromo-2,3,4,5,6-pentafluorotoluene and *N,N*-disopropylethylamine were added to esterify the acid groups and *N,O-bis*(trimethylsilyl)trifluoroacetamide with trimethylchlorosilane was used to derivatize the hydroxy groups. The derivatives were injected into a Varian model 3400 gas chromatograph and detected by a Finnigan SSQ7000 mass spectrometer, set for selected ion monitoring for each compound. The quantitation limit for both simvastatin acid and pravastatin was 0.5 ng/ml. Itraconazole did not interfere with the GC-MS assay. (A more detailed method description can be obtained by writing to the corresponding author, P.J.N.)

Radioenzyme inhibition assay of HMG-CoA reductase inhibitors. Serum concentrations of HMG-CoA reductase inhibitors were determined with use of radioenzyme inhibition assay[17,18] after base hydrolysis of lactones. Base hydrolysis was accomplished by incubating 0.1 ml serum with 0.01 ml of 1.0N

Table I. Characteristics of the subjects and pharmacokinetic parameters of itraconazole and hydroxyitraconazole on day 4 of 200 mg/day itraconazole administration (i.e., on the day of simvastatin [study I] or pravastatin [study II] administration)

Subject No.	Sex	Age (yr)	Weight (kg)	Itraconazole C_{max} (ng · ml^{-1})	t_{max} (hr)	C_{min} (ng · ml^{-1})	$t_{1/2}$ (hr)	AUC(0-24) (ng · ml^{-1} · hr)	Hydroxyitraconazole C_{max} (ng · ml^{-1})	t_{max} (hr)	C_{min} (ng · ml^{-1})	$t_{1/2}$ (hr)	AUC(0-24) (ng · ml^{-1} · hr)
Study I: Simvastatin													
1	Male	22	72	654	4	210	28.8	9,475	1,160	4	758	64.6	24,699
2*	Female	21	57	409	3	91	17.1	4,303	596	3	237	20.0	10,300
3	Male	21	75	214	4	61	16.7	2,790	462	4	200	18.0	7,846
4	Male	19	80	549	4	143	31.8	6,850	842	4	460	80.4	16,350
5	Male	29	79	584	1	171	21.0	7,249	823	4	541	35.3	16,024
6	Male	29	77	331	1	152	35.6	5,084	626	6	410	32.7	12,840
7*	Female	20	55	773	3	147	22.0	7,517	935	4	388	26.4	16,255
8	Male	21	70	640	2	184	18.2	7,931	979	4	595	34.9	18,334
9	Female	20	57	536	2	97	15.4	4,763	851	3	276	13.4	12,402
10	Male	23	69	398	3	126	21.8	5,055	704	4	355	44.7	15,154
Mean		22.5	69.1	509	2.7	138	22.8	6,101	798	4.0	422	37.0	15,020
SEM		1.1	3.0	53	0.4	14	2.2	639	65	0.3	55	6.7	1,469
Study II: Pravastatin													
11	Male	21	62	411	4	141	18.2	6,020	697	5	451	28.5	14,251
12	Male	23	61	498	2	134	30.1	6,875	819	4	410	43.4	17,072
13	Male	20	65	531	3	157	19.1	7,273	770	12	327	63.8	16,416
14*	Female	23	52	997	2	245	34.9	10,288	1,120	3	580	55.8	20,662
15*	Female	21	65	655	3	137	18.3	6,785	931	3	432	25.2	16,178
16	Male	19	65	293	2	58	11.4	3,559	594	4	211	13.2	9,804
17	Male	23	86	244	0.5	55	11.6	3,128	609	4	251	16.3	10,599
18	Male	19	77	259	2	54	14.4	2,845	461	2	168	15.1	7,404
19	Male	22	72	509	4	133	13.0	6,379	745	4	290	26.9	14,445
20*	Female	22	56	880	3	258	35.1	9,359	1,170	3	729	65.0	22,906
Mean		21.3	66.1	528	2.6	137	20.6	6,251	792	4.4	385	35.3	14,974
SEM		0.5	3.2	81	0.3	23	2.9	791	72	0.9	55	6.4	1,517

C_{max}, Peak serum concentration; t_{max}, time to reach C_{max}; C_{min}, minimum serum concentration; $t_{1/2}$, elimination half-life; AUC(0-24), area under the serum concentration–time curve from 0 to 24 hours.

*Subjects were using contraceptive steroids. Subjects 2 and 14 were taking 2 mg cyproterone acetate plus 35 μg ethinyl estradiol (INN, ethinylestradiol); subjects 7 and 20 were taking 0.15 mg desogestrel plus 20 μg ethinyl estradiol; subject 15 was taking 75 μg gestodene plus 30 μg ethinyl estradiol.

potassium hydroxide at room temperature for 15 minutes. Concentrations are reported as nanogram equivalents of simvastatin acid per milliliter (study I) or nanogram equivalents of pravastatin sodium per milliliter (study II). The quantitation limit for simvastatin acid and pravastatin was 2 and 5 ng/ml, respectively. The CV was 7.0% for simvastatin (at 11.1 ng-eq/ml; $n = 6$) and 4.0% for pravastatin (at 10.5 ng-eq/ml; $n = 6$). Itraconazole did not interfere with the assay.

Determination of itraconazole and hydroxyitraconazole. The concentrations of itraconazole and hydroxyitraconazole were determined by HPLC with use of fluorescence detection.[7,19] The mobile phase was water/acetonitrile (50:50) and contained 0.28% of triethylamine. The detection limit was 10 ng/ml. The CV was 1.6% for itraconazole (at 194 ng/ml; $n = 6$) and 1.2% for hydroxyitraconazole (at 194 ng/ml; $n = 6$).

Pharmacokinetic calculations. The pharmacokinetics were characterized by peak concentration (C_{max}) in serum (and minimum serum concentration [C_{min}] for itraconazole and hydroxyitraconazole), time to peak concentration (t_{max}), elimination half-life ($t_{1/2}$), and areas under the serum concentration-time curve up to 24 hours [AUC(0-24)] and to infinity [AUC(0-∞)]. The elimination rate constant (k_e) was determined by a linear regression analysis of the log-linear phase of the serum drug concentration–time curve. The $t_{1/2}$ was calculated from $t_{1/2} = \ln 2/k_e$. The AUC(0-∞) was calculated by use of the trapezoidal rule with extrapolation to infinity by dividing the last measured concentration by k_e. The AUC(0-24) values of itraconazole and hydroxy-

itraconazole refer to the time between 0 and 24 hours after the ingestion of simvastatin or pravastatin (i.e., between 2 and 26 hours after the last dose of itraconazole).

Statistical analysis. All data are expressed as mean values ± SEM. Data were analyzed by the Student *t* test for paired values (two-tailed), except for t_{max} data, which were analyzed by the Wilcoxon test. Differences were regarded as statistically significant when p values were <0.05.

RESULTS

Study I: Simvastatin

Simvastatin. During the placebo phase, serum concentrations of unchanged simvastatin were below the detection limit (<10 ng/ml by HPLC), but itraconazole greatly increased serum simvastatin concentrations (Fig. 1). During the itraconazole phase, both the mean C_{max} (154 ± 22 ng/ml) and the AUC(0-∞) values (553 ± 52 ng/ml hr) of simvastatin were at least tenfold greater ($p < 0.001$) than those during the placebo phase.

Simvastatin acid. Itraconazole increased the mean C_{max} of total simvastatin acid 17-fold ($p < 0.001$) and the AUC(0-∞) 19-fold ($p < 0.001$; Fig. 1 and Table II). The mean $t_{1/2}$ was increased by only 25% ($p < 0.05$) by itraconazole. The ratio of the C_{max} in the itraconazole phase to the C_{max} in the placebo phase ranged from 7 to 56 and the corresponding AUC(0-∞) ratio from 13 to 25 (Fig. 2 and Table II). The interaction was greatest in subjects 1 and 8 who also showed the highest concentrations of itraconazole and hydroxyitraconazole (Table I).

During the itraconazole phase, the mean C_{max} (86 ± 33 ng/ml) and the AUC(0-∞) values (425 ± 104 ng/ml · hr) of simvastatin acid (i.e., total simvastatin acid minus simvastatin) were at least tenfold greater ($p < 0.001$) than those during the placebo phase (the exact values could not be calculated during the placebo phase).

HMG-CoA reductase inhibitors. Itraconazole considerably increased the concentration of HMG-CoA reductase inhibitors in serum (Fig. 1). During the itraconazole phase, the mean AUC(0-∞) was more than fivefold ($p < 0.001$) greater than that after the ingestion of simvastatin during the placebo phase. The mean C_{max} and $t_{1/2}$ values of the HMG-CoA reductase inhibitors were about threefold ($p < 0.001$) greater during the itraconazole than those during the placebo phase (Table III).

Study II: Pravastatin

Pravastatin. Although itraconazole seemed to increase the mean AUC(0-∞) and C_{max} of pravastatin, the changes were not statistically significant ($p = 0.052$ and 0.172, respectively) (Fig. 3 and Table IV). In contrast to the great individual increases in the AUC(0-∞) of the total simvastatin acid caused by itraconazole, the individual changes in the AUC(0-∞) of pravastatin were relatively small, and decreases in the AUC(0-∞) even occurred (Fig. 2). In one subject (subject 20), both the C_{max} and the AUC(0-∞) values of pravastatin were ninefold higher during the itraconazole phase than those during the placebo phase. Her C_{max} and AUC(0-∞) values for hydroxyitraconazole were also somewhat higher than those in the other subjects (Table I). The $t_{1/2}$ of pravastatin was not altered by itraconazole.

HMG-CoA reductase inhibitors. The mean AUC(0-∞) and C_{max} of HMG-CoA reductase inhibitors after ingestion of pravastatin were increased by less than twofold ($p < 0.05$ and $p = 0.063$, respectively) by itraconazole (Fig. 3 and Table III). The $t_{1/2}$ was not affected by itraconazole.

Itraconazole and hydroxyitraconazole

The serum concentrations and pharmacokinetic parameters of itraconazole and hydroxyitraconazole in study I and study II were practically identical (Fig. 4 and Table I). The AUC(0-24) of hydroxyitraconazole was twofold to threefold higher than that of itraconazole in both studies. Furthermore, in both studies there were interindividual differences of about fourfold in the C_{max}, C_{min}, and AUC(0-24) values of itraconazole and hydroxyitraconazole.

DISCUSSION

These results indicate that itraconazole greatly increases serum concentrations of simvastatin, simvastatin acid, and HMG-CoA reductase inhibitors. In contrast to this, the same dose of itraconazole had only minor effects on the AUC of pravastatin and HMG-CoA reductase inhibitors after the ingestion of pravastatin.

All volunteers in this study were young and healthy. Although five women used contraceptive steroids, the results obtained in those subjects did not differ from the results obtained in the other subjects, and both volunteer groups were nearly identical with respect to, for example, the pharmacokinetics of itraconazole and hydroxyitraconazole, as shown in Table I and Fig. 4. Thus the randomized

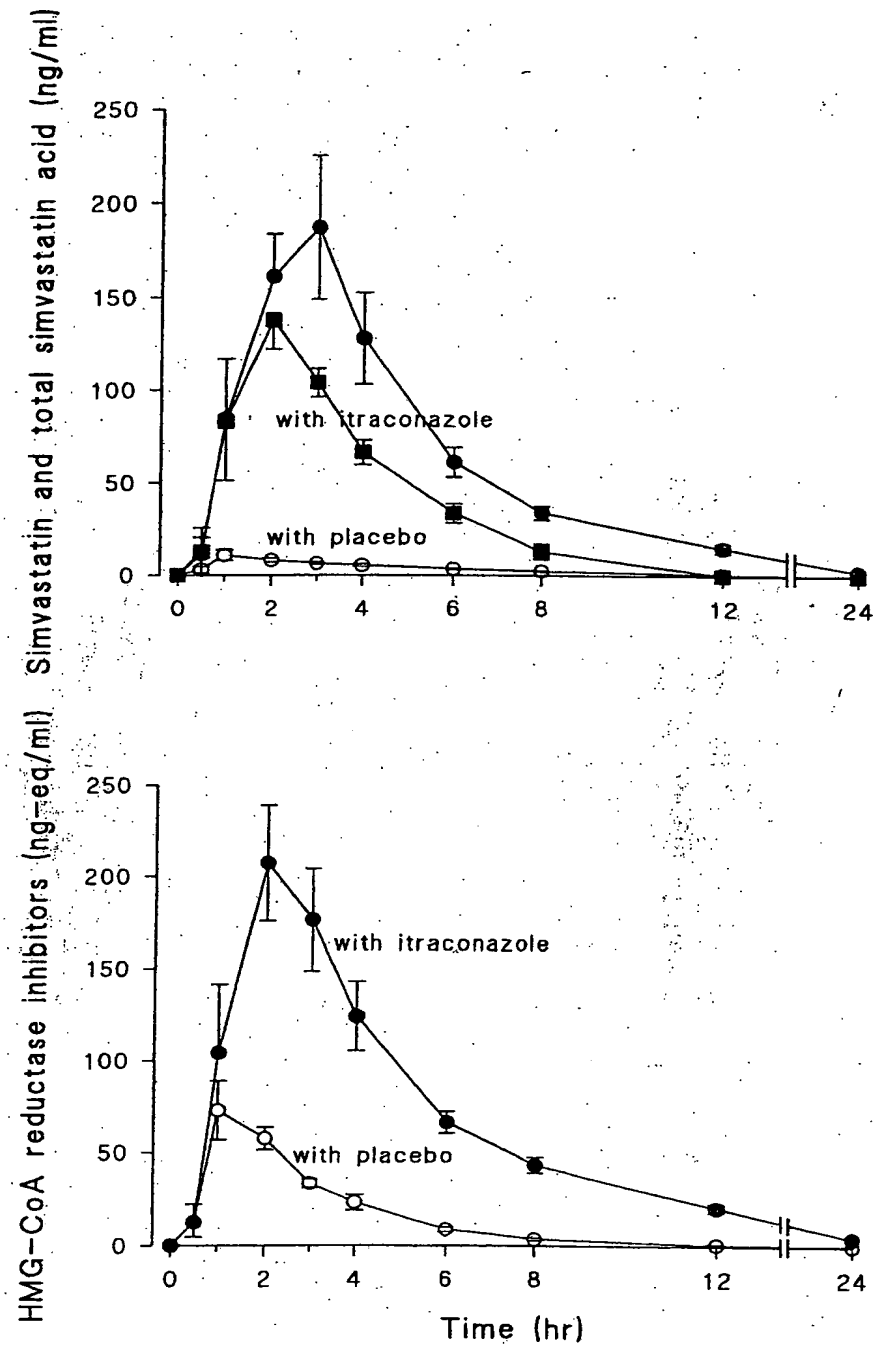

Fig. 1. Mean ± SEM concentrations of unchanged simvastatin *(squares)*, total simvastatin acid *(circles)* **(upper panel)**, and total 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors **(lower panel)** in serum of 10 healthy volunteers after a single oral dose of 40 mg simvastatin after daily use of oral itraconazole (200 mg; *solid squares* and *solid circles*) or placebo *(open circles)* for 4 days. The concentrations of unchanged simvastatin were undetectable during the placebo phase. Ng-eq refers to the nanogram equivalents of simvastatin acid.

Table II. C_{max}, t_{max}, $t_{1/2}$, and AUC(0-∞) of total simvastatin acid (study I) during the itraconazole and placebo phases*

Subject No.	C_{max} (ng · ml^{-1})			t_{max} (hr)		$t_{1/2}$ (hr)			AUC(0-∞) (ng · ml^{-1} · hr)		
	Itraconazole	Placebo	Ratio	Itraconazole	Placebo	Itraconazole	Placebo	Ratio	Itraconazole	Placebo	Ratio
1	499	8.9	56	3	4	4.0	4.7	0.9	1,827	74	25
2	233	25	9	2	1	3.8	2.4	1.6	981	51	19
3	95	5.3	18	2	1	3.5	3.1	1.1	522	33	16
4	215	11	20	3	2	5.1	2.9	1.8	996	51	20
5	131	19	7	1	1	4.8	3.4	1.4	726	54	13
6	94	7.5	13	3	1	3.0	3.5	0.9	563	42	13
7	355	26	14	1	1	3.7	2.4	1.5	1,402	72	19
8	139	6.0	23	2	2	3.2	2.8	1.1	725	32	23
9	188	10	19	3	3	4.8	2.8	1.7	1,069	50	21
10	217	11	20	2	1	3.0	3.0	1.0	961	66	15
Mean	217†	13.0	19	2.2	1.7	3.9‡	3.1	1.3	977†	52.5	19
SEM	40	2.4		0.3	0.3	0.2	0.2		126	4.7	

AUC(0-∞), Area under the serum concentration–time curve from time 0 to infinity.
*A 40 mg dose of simvastatin was taken during both the itraconazole and placebo phases.
†Significantly different from the placebo phase, $p < 0.001$.
‡Significantly different from the placebo phase, $p < 0.05$.

Table III. C_{max}, t_{max}, $t_{1/2}$, and AUC(0-∞) of HMG-CoA reductase inhibitors after ingestion of a single 40 mg dose of simvastatin (study I) or pravastatin (study II) during the itraconazole and placebo phases*

	C_{max} (ng-eq · ml^{-1})			t_{max} (hr)		$t_{1/2}$ (hr)			AUC(0-∞) (ng-eq · ml^{-1} · hr)		
	Itraconazole	Placebo	Ratio	Itraconazole	Placebo	Itraconazole	Placebo	Ratio	Itraconazole	Placebo	Ratio
Study I: Simvastatin											
Mean	234†	85	2.7	2.1	1.6	4.4†	1.6	2.8	1,133†	219	5.2
SEM	35	12		0.2	0.3	0.3	0.1		114	16	
Study II: Pravastatin											
Mean	82	49	1.7	1.3	1.3	1.3	1.2	1.1	189‡	111	1.7
SEM	23	12		0.2	0.2	0.1	0.1		49	32	

HMG-CoA, 3-Hydroxy-3-methylglutaryl coenzyme A.
*ng-eq refers to the nanogram equivalents of simvastatin acid (study I) and pravastatin sodium (study II), respectively.
†Significantly different from the placebo phase, $p < 0.001$.
‡Significantly different from the placebo phase, $p < 0.05$.

Table IV. C_{max}, t_{max}, $t_{1/2}$, and AUC(0-∞) of pravastatin (study II) during the itraconazole and placebo phases*

Subject No.	C_{max} (ng · ml^{-1})			t_{max} (hr)		$t_{1/2}$ (hr)			AUC(0-∞) (ng · ml^{-1} · hr)		
	Itraconazole	Placebo	Ratio	Itraconazole	Placebo	Itraconazole	Placebo	Ratio	Itraconazole	Placebo	Ratio
11	41	32	1.3	2	1	1.8	1.6	1.1	113	65	1.7
12	24	13	1.8	1	2	1.5	2.4	0.6	62	44	1.4
13	33	13	2.5	1	1	1.4	1.6	0.9	79	31	2.5
14	135	146	0.9	1	1	1.7	1.7	1.0	297	378	0.8
15	26	31	0.8	2	1	1.3	1.1	1.2	65	70	0.9
16	54	10	5.4	1	1	2.5	2.5	1.0	128	35	3.7
17	12	21	0.6	2	1	3.4	1.1	3.1	56	35	1.6
18	73	34	2.1	0.5	1	1.9	2.0	1.0	163	99	1.6
19	16	43	0.4	2	1	1.3	1.8	0.7	44	70	0.6
20	250	26	9.6	1	2	1.6	1.2	1.3	512	59	8.7
Mean	66.4	36.9	2.5	1.4	1.2	1.8	1.7	1.1	152.0†	88.6	1.7
SEM	23.4	12.6		0.2	0.1	0.2	0.2		46.5	32.9	

*A 40 mg dose of pravastatin was taken during both the itraconazole and placebo phases.
†After log transformation, $p = 0.052$ compared with the placebo phase.

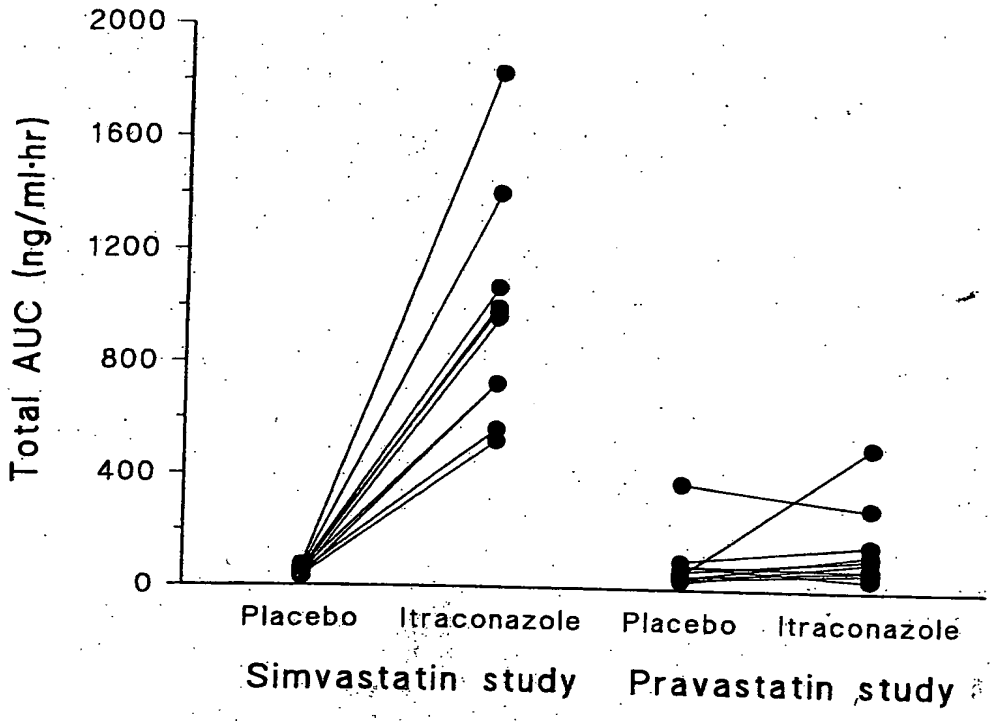

Fig. 2. Individual changes in the values of the area under the concentration-time curve to infinity [AUC(0-∞)] of total simvastatin acid (study I, simvastatin study) and those of pravastatin (study II, pravastatin study) caused by itraconazole.

crossover design used allows a comparison of the effect of itraconazole both within and between the groups. Because of the long elimination $t_{1/2}$ values of itraconazole and hydroxyitraconazole, the use of a four-phase crossover study design, instead of two separate studies, would have lasted for 3 months and carried a great risk of drop-outs.

The sensitivity of the GC-MS method used is about tenfold better than that of the HPLC method, allowing quantitation of serum pravastatin and total simvastatin acid concentrations also during the placebo phase. However, the GC-MS analysis necessitates a derivatization that is not possible to do to the parent simvastatin lactone. Accordingly, total simvastatin acid includes both naive simvastatin acid and simvastatin that was converted to the acid form. The concentrations of unchanged simvastatin were determined by the HPLC method; that is, the difference between the values for total simvastatin acid and simvastatin represents naive simvastatin acid. The measurement of the HMG-CoA reductase inhibitors by radioenzyme inhibition assay included all active HMG-CoA reductase inhibitors (i.e., pravastatin, simvastatin acid, and all active metabolites) and those activated by alkaline hydrolysis.

The systemic bioavailability of simvastatin is less than 10%, the major part of simvastatin being metabolized during the first pass.[20] Simvastatin itself is an inactive lactone pro-drug, converted reversibly to the active HMG-CoA reductase inhibitor simvastatin acid by esterases and even nonenzymatically. Most of the other metabolites are formed by CYP3A enzymes, and they are inactive or weak HMG-CoA reductase inhibitors; however, 6-hydroxymethylsimvastatin and simvastatin 6-carboxylic acid have 90% and 40% of the activity of simvastatin acid, respectively.[10,20]

Itraconazole seems to interact with simvastatin in a manner very similar to that of lovastatin,[7] which also is a lipophilic substrate of CYP3A and has a low bioavailability. Their interaction with itraconazole probably takes place already during the first pass. The duodenal wall mucosa, where CYP3A4 is extensively expressed,[21,22] seems to be an important site of interaction because the $t_{1/2}$ values (clearances) have been

affected to only a small extent, despite greatly increased C_{max} and AUC values.

Itraconazole increased the AUC(0-∞) and C_{max} values of simvastatin and total simvastatin acid about tenfold to twentyfold, whereas those of the HMG-CoA reductase inhibitors were increased less (i.e., fivefold and threefold, respectively). Thus itraconazole seems to reduce the CYP3A4-mediated formation of both active and inactive metabolites of simvastatin during the first pass, thereby increasing the bioavailability of simvastatin more than that of (total) HMG-CoA reductase inhibitors. On the other hand, itraconazole clearly increased the $t_{1/2}$ of the HMG-CoA reductase inhibitors more than that of the total simvastatin acid (i.e., by 300% and 25%, respectively).

In contrast to simvastatin, which is a pro-drug, pravastatin is an active HMG-CoA reductase inhibitor. The bioavailability of pravastatin averages 18%, and it is excreted to a significant extent as the parent drug into urine.[11,23] Pravastatin is biotransformed by several enzymes and the metabolites have only a minor activity.[11,24,25] In vitro, pravastatin itself has been found to be a rather weak competitive inhibitor of CYP3A4.[26] In the present study, itraconazole had only a small effect on the pharmacokinetics of pravastatin or of HMG-CoA reductase inhibitors after pravastatin ingestion. These findings suggest that CYP3A4 plays only a small role in the metabolism of pravastatin.

Pravastatin is more hydrophilic than simvastatin and therefore it does not easily cross cell membranes. However, HMG-CoA reductase inhibitors are also taken up into cells by transport mechanisms and the P-glycoprotein transporter is present, for example, in intestinal microvilli. Theoretically, at least, inhibitors of the P-glycoprotein transporter, such as itraconazole and cyclosporine, could also interact with pravastatin and other HMG-CoA reductase inhibitors by this mechanism.[8]

Because of the dose-dependent toxicity of HMG-CoA reductase inhibitors, use of simvastatin or lovastatin at conventional dosages together with potent inhibitors of CYP3A4 carries an enhanced risk of skeletal muscle toxicity. In addition to itraconazole, other drugs, such as ketoconazole, cyclosporine, erythromycin, clarithromycin, verapamil, mibefradil, and diltiazem, are significant inhibitors of CYP3A4 (and of P-glycoprotein). Recently 120 mg/day diltiazem was reported to increase the C_{max} and AUC of lovastatin about fourfold, without a significant effect on the pharmacokinetics of pravastatin.[27]

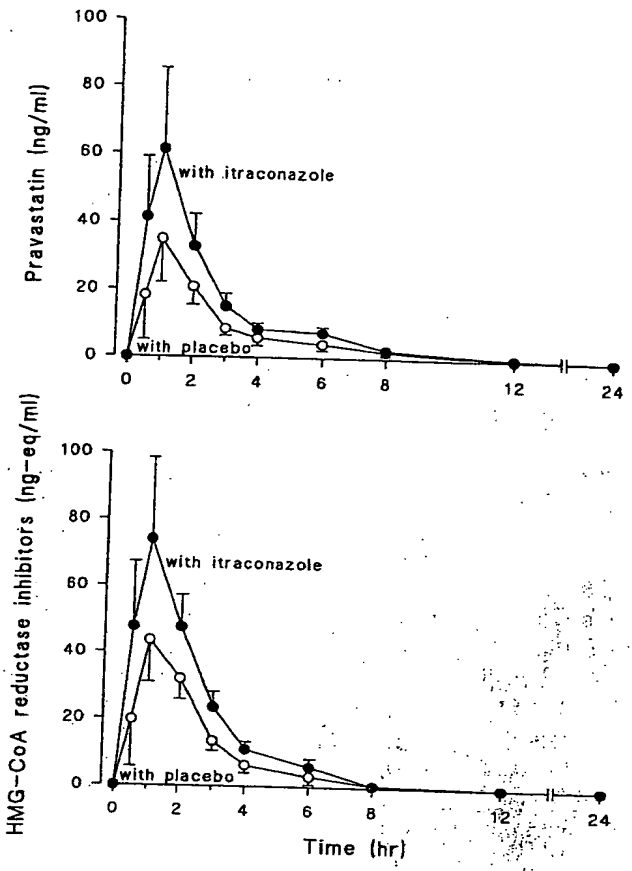

Fig. 3. Mean ± SEM concentrations of unchanged pravastatin **(upper panel)** and total HMG-CoA reductase inhibitors **(lower panel)** in serum of 10 healthy volunteers after a single oral dose of 40 mg pravastatin after daily use of oral itraconazole (200 mg; *solid circles*) or placebo *(open circles)* for 4 days. Ng-eq refers to the nanogram equivalents of pravastatin sodium.

Elevated serum concentrations of simvastatin and pravastatin have been reported in organ transplant patients taking many drugs, including cyclosporine, compared with patients who were not receiving cyclosporine.[12-13] However, the interpretation of these findings with regard to drug interactions is difficult; underlying diseases themselves (e.g., decreased renal, hepatic, or cardiac function in organ transplant patients) may have significant effects on the pharmacokinetics of HMG-CoA reductase inhibitors.

In this study the interaction caused by itraconazole seemed to be greatest in those subjects who had the highest concentrations of serum itracon-

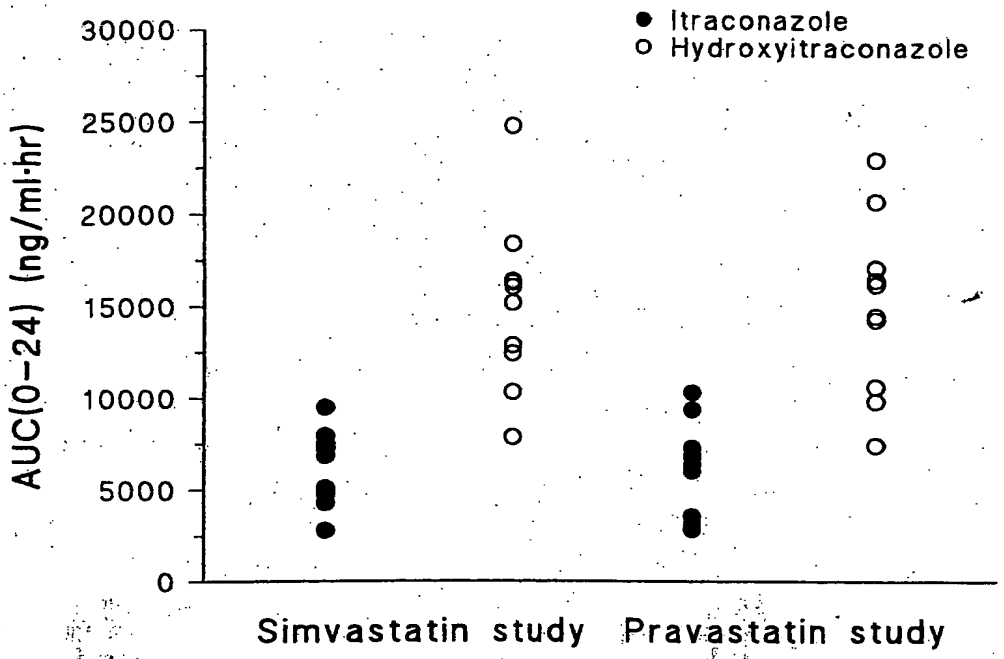

Fig. 4. The individual areas under the concentration-time curve up to 24 hours [AUC(0-24)] of itraconazole and hydroxyitraconazole in the 10 subjects participating in the simvastatin study (study I) and those in the 10 subjects participating in the pravastatin study (study II).

azole and hydroxyitraconazole. The subjects ingested itraconazole at a moderate dosage of 200 mg daily for 4 days before the ingestion of simvastatin or pravastatin. For example, in the oral treatment of nail mycoses, itraconazole is used for several weeks and its steady-state concentrations are often twofold higher than the concentrations in our study. Accordingly, it is possible that the interaction could be greater after a more prolonged use of higher doses of itraconazole.

It may be concluded that itraconazole interacts with simvastatin to a great extent, probably by preventing its CYP3A4-mediated metabolism. In contrast, itraconazole showed only a minor interaction with pravastatin. Concomitant use of potent inhibitors of CYP3A4 with simvastatin or lovastatin should be avoided or the dosages of these HMG-CoA reductase inhibitors should be greatly reduced. Pravastatin is less susceptible to this kind of an interaction.

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References

1. Scandinavian Simvastatin Survival Study Group. Randomized trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S). Lancet 1994;344:1383-9.
2. Sacks FM, Pfeffer MA, Moye LA, Rouleau JL, Rutherford JD, Cole TG, et al. The effect of pravastatin on coronary events after myocardial infarction in patients with average cholesterol levels. N Engl J Med 1996; 335:1001-9.
3. Norman DJ, Illingworth DR, Munson J, Hosenpud J. Myolysis and acute renal failure in a heart-transplant patient receiving lovastatin [letter]. N Engl J Med 1988;318:46-7.
4. Lees RS, Lees AM. Rhabdomyolysis from the coadministration of lovastatin and the antifungal agent itraconazole [letter]. N Engl J Med 1995;333:664-5.
5. Segaert MF, De Soete C, Vandewiele I, Verbanck J. Drug interaction–induced rhabdomyolysis. Nephrol Dial Transplant 1996;11:1846-7.
6. Horn M. Coadministration of itraconazole with hypolipidemic agents may induce rhabdomyolysis in healthy individuals [letter]. Arch Dermatol 1996;132: 1254.
7. Neuvonen PJ, Jalava KM. Itraconazole drastically increases plasma concentrations of lovastatin and lovastatin acid. Clin Pharmacol Ther 1996;60:54-61.

8. Bertz RJ, Granneman GR. Use of in vitro and in vivo data to estimate the likelihood of metabolic pharmacokinetic interactions. Clin Pharmacokinet 1997;32: 210-58.
9. Wang RW, Kari PH, Lu AYH, Thomas PE, Guengerich FP, Vyas KP. Biotransformation of lovastatin; IV: identification of cytochrome P-450 3A proteins as the major enzymes responsible for the oxidative metabolism of lovastatin in rat and human liver microsomes. Arch Biochem Biophys 1991;290:355-61.
10. Vickers S, Duncan CA, Vyas KP, Kari PH, Arison B, Prakash SR, et al. In vitro and in vivo biotransformation of simvastatin, an inhibitor of HMG CoA reductase. Drug Metab Dispos 1990;18:476-83.
11. Haria M, McTavish D. Pravastatin: a reappraisal of its pharmacological properties and clinical effectiveness in the management of coronary heart disease. Drugs 1997;53:299-336.
12. Arnadottir M, Eriksson LO, Thysell H, Karkas JD. Plasma concentration profiles of simvastatin 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase inhibitory activity in kidney transplant recipients with and without ciclosporin. Nephron 1993;65:410-3.
13. Kliem V, Wanner C, Eisenhauer T, Olbricht CJ, Doll R, Boddaert M, et al. Comparison of pravastatin and lovastatin in renal transplant patients receiving cyclosporine. Transplant Proc 1996;28:3126-8.
14. Stubbs RJ, Schwartz M, Bayne WF. Determination of mevinolin and mevinolinic acid in plasma and bile by reversed-phase high-performance liquid chromatography. J Chromatogr 1986;383:438-43.
15. Morris MJ, Gilbert JD, Hsieh JY, Matuszewski BK, Ramjit HG, Bayne WF. Determination of the HMG-CoA reductase inhibitors simvastatin, lovastatin, and pravastatin in plasma by gas chromatography/chemical ionization mass spectrometry. Biol Mass Spectrom 1993;22:1-8.
16. Funke PT, Ivashkiv E, Arnold ME, Cohen AI. Determination of pravastatin sodium and its major metabolites in human serum/plasma by capillary gas chromatography/negative ion chemical ionization mass spectrometry. Biomed Environ Mass Spectrom 1989; 18:904-9.
17. Manning JA, Swanson BN, Emaus NA, Fry J. A semiautomatic, radioenzymatic assay for pravastatin and lovastatin in human serum [abstract]. Pharm Res 1984;6:237.
18. Pentikäinen PJ, Saraheimo M, Schwartz JI, Amin RD, Schwartz MS, Brunner-Ferber F, et al. Comparative pharmacokinetics of lovastatin, simvastatin and pravastatin in humans. J Clin Pharmacol 1992;32:136-40.
19. Allenmark S, Edebo A, Lindgren K. Determination of itraconazole in serum with high-performance liquid chromatography and fluorescence detection. J Chromatogr 1990;532:203-6.
20. Vickers S, Duncan CA, Chen IW, Rosegay A, Duggan DE. Metabolic disposition studies on simvastatin, a cholesterol-lowering prodrug. Drug Metab Dispos 1990;18:138-45.
21. Kolars JC, Lown KS, Schmiedlin-Ren P, Ghosh M, Fang C, Wrighton SA, et al. CYP3A gene expression in human gut epithelium. Pharmacogenetics 1994;4: 247-59.
22. Kivistö KT, Bookjans G, Fromm MF, Griese EU, Munzel P, Kroemer HK. Expression of CYP3A4, CYP3A5 and CYP3A7 in human duodenal tissue. Br J Clin Pharmacol 1996;42:387-9.
23. Singhvi SM, Pan HY, Morrison RA, Willard DA. Disposition of pravastatin sodium, a tissue-selective HMG-CoA reductase inhibitor, in healthy subjects. Br J Clin Pharmacol 1990;29:239-43.
24. Kitazawa E, Tamura N, Iwabuchi H, Uchiyama M, Muramatsu S, Takahagi H, et al. Biotransformation of pravastatin sodium; I: mechanism of enzymic transformation of epimerization of an allylic hydroxy group of pravastatin sodium. Biochem Biophys Res Commun 1993;192:597-602.
25. Muramatsu S, Miyaguchi K, Iwabuchi H, Matsushita Y, Nakamura T, Kinoshita T, et al. Metabolism of pravastatin sodium in isolated rat hepatocytes. Xenobiotica 1992;22:487-98.
26. Transon C, Leemann T, Dayer P. In vitro comparative inhibition profiles of major human drug metabolising cytochrome P450 isozymes (CYP2C9, CYP2D6 and CYP3A4) by HMG-CoA reductase inhibitors. Eur J Clin Pharmacol 1996;50:209-15.
27. Agbin NE, Brater DC, Hall SD. Interaction of diltiazem with lovastatin and pravastatin [abstract]. Clin Pharmacol Ther 1997;61:201.